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WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

No. 165

**EXPERT COMMITTEE ON
PLAGUE**

Third Report

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WORLD HEALTH ORGANIZATION

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GENEVA

1959

EXPERT COMMITTEE ON PLAGUE

Geneva, 15-20 September 1958

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EXPERT COMMITTEE ON PLAGUE

Third Report *

Dr Karl F. Meyer was unanimously elected Chairman; Dr M. Baltazard and Dr R. B. Heisch were elected Rapporteurs.

The provisional agenda was adopted with some alterations.

EPIDEMIOLOGY

Epidemiological studies on wild rodent plague

The various epidemiological patterns of plague throughout the world were compared. In Africa the most striking recent advance is the discovery of wild rodent plague in Kenya. This has changed the previous ideas on the epidemiology of plague in Central Africa, where it was formerly believed that *Rattus rattus* was the only primary reservoir.

Apart from *R. rattus*, the chief rodents involved were *Otomys*, *Arvicanthhis* and *Mastomys coucha* (*Rattus natalensis*). The main flea vectors were *Xenopsylla cheopis* and *Dinopsyllus ellobius typlusus*.

An important aspect of these investigations was that wild rodents were found infected in the bush far from human habitations. This suggests that the wild rodents might be the main reservoir of *Pasteurella pestis*, the more so as there is evidence of resistance to plague in the three wild rodents involved. It was pointed out, however, that the presence of the infection in them is usually a temporary phenomenon and that permanent foci of plague in these animals have yet to be discovered.

Concerning the Belgian Congo, it was stated that for the last 20 years systematic deratting followed by examination of the pooled bone-marrow of the animals was carried out, about 1000 rodents being tested daily. On an average, one out of 5000-6000 rodents proved positive. In the Blukwa focus the multimammate mouse (*R. natalensis*) was the only commensal rodent, whereas in Kivu this species co-existed with *R. rattus* in the huts.

* The Executive Board, at its twenty-third session, adopted the following resolution:
The Executive Board

1. NOTES the third report of the Expert Committee on Plague;
2. THANKS the members of the Committee for their work; and
3. AUTHORIZES publication of the report.

(Resolution EB23.R29, *Off. Rec. Wld Hlth Org.*, 1959, 91, 17)

X. cheopis and *X. brasiliensis* were met with in the Blukwa focus. In the Kivu area only *X. brasiliensis* was found and was responsible for the transmission of plague to man.

Studies carried out in the outskirts of the infected villages yielded evidence of the existence of plague in wild rodents. The prevailing species were *Arvicanthis* and, to a lesser extent, *Otomys*. In contrast to *Mastomys* and *Arvicanthis*, *Otomys* was markedly susceptible to plague. In the Blukwa area, *Dinopsyllus lypusus* was present and proved a vector of the infection. In the Kivu focus, several *Dinopsyllus* species were met with, but their role is still obscure. *Ctenophthalmus* was also present but did not serve as a plague vector. *Ctenocephalides felis*, and not *Pulex irritans*, was found on man, but experimentally did not transmit the infection. The fatality rate from human plague was very high.

In Kenya, *Otomys* seems to be more resistant to plague. It seemed to be very important in perpetuating the infection, while *Arvicanthis*, though also involved, tended to disappear during the inter-epidemic periods. *Dinopsyllus lypusus*, while frequent on the wild species, was rare on *Rattus*. In a plague-infected village high up on the forest edge, *P. irritans* was the common flea species on man. So far this species has not been found plague-infected.

In the Soviet Union, *P. irritans* was found to be a vector in those areas where mice harboured *P. pestis*.

In South America, transmission experiments failed to implicate *P. irritans* as a vector of plague.

Interesting new advances have been reported from Iranian Kurdistan. It was formerly believed that only three species of *Meriones* were present in the focus and that all were resistant to plague. It is now known that *M. tristrami* is not a sub-species of *M. shawi*, but an independent species existing in the area under investigation. It was also demonstrated that *M. libycus erythourus* included two species, one of which was *M. libycus erythourus* and the other *M. vinogradovi*. Of these species, *M. tristrami* and *M. vinogradovi* are extremely susceptible to plague. This makes it clear that in the plague focus of Kurdistan there are not only resistant wild rodents (*M. persicus* and *M. libycus*), but susceptible ones (*M. tristrami* and *M. vinogradovi*).¹

The persistence of plague in Kurdistan thus depends on the ecological interplay between susceptible and resistant rodent species living in close contact.

Further work in Turkey, Syria and Iraq has failed to confirm the existence of sylvatic plague. However, it is noteworthy that one of the human plague strains isolated near the Syrian border in Turkey had the same biochemical

¹ Reservoirs are given in Annex 1, page 16.

characters as the Iranian and south-west Russian strains (glycerol-positive and nitrite-negative).

These observations tend to support the view that these four foci form part of a large enzootic area where commensal rats are absent.

Evidence that field rodents play a primary role has also been adduced in the northern provinces of India. Of the common species involved, one, *Tatera indica*, is markedly resistant to the infection, while *Millardia* and *Bandicota* are markedly susceptible.

It has been demonstrated that there are no permanent foci of plague as in other parts of Asia and in Africa, but that the infection is constantly on the move, affecting the villages on a serpentine course through the fields.

Although, owing to widespread inundations during the monsoon, most of the rodents living in the low-lying parts of the area are drowned, those inhabiting higher ground survive. These rodents multiply rapidly after the floods have subsided and their progeny become infected from the fleas¹ which have carried over the infection while subsisting on the resistant rodent species (*Tatera*) residing in the deep, closed burrows. The fact that "endemic" areas in north India are mostly situated in districts which are least liable to inundations supports the above concept. It should also be pointed out that there is a marked predominance of *Tatera indica* in the "endemic" areas. However, this rodent is neither resistant nor sedentary enough to ensure the existence of true permanent pockets of infection.

Observations in urban centres, particularly in Bombay and Calcutta, indicate a steadily declining incidence of the commensal rat species, compensated for by an increased frequency of *Bandicota bengalensis*. The rodent density in the different wards of Calcutta continues to vary considerably.

Experiments in Calcutta undertaken with the aid of differently dyed foodstuffs confirmed that in that city the rats were rather sedentary, the range of their movements not exceeding 200 yards. Tattooed rats released after they had been moved from distant wards of the city, showed a tendency to return to their habitat.

Epidemiological observations in Calcutta showed that proximity of plague-infected rats was essential for the appearance of human infection.

Transport of goods in boats and barges on the Hooghli River within the city limits of Calcutta appeared to play a small part in the spread of plague.

Researches in Java, using techniques like those employed in Kurdistan and the northern provinces of India, have demonstrated a similar epidemiological pattern. The permanent reservoir is a rodent now identified as *Rattus exulans* which, like *Tatera*, is markedly resistant to plague and inhabits deep burrows in dry ground. The distribution of this rat coincides with the areas of endemicity.

¹ See Annex 2, page 29.

Extensive investigations on the ecology of plague in the Soviet Union have confirmed the existence of four main foci of infection, situated in the Pre-Caspian area, Central Asia, the Transcaucasus, and Transbaikalia. Altogether, about 40 species of rodents have been found infected, among which the following are considered as primary reservoirs of the infection:

<i>Species</i>	<i>Area</i>
<i>Citellus pygmaeus</i>	Pre-Caspian area
<i>Rhombomys opimus</i> , <i>Marmota baibacina</i> , <i>Marmota caudata</i>	Central Asia
<i>Meriones erythrorus</i>	Transcaucasus
<i>Marmota sibirica</i>	Transbaikalia

Commensal mice can serve as carriers of the infection, while the other rodents play only an occasional role. Rats are absent in the endemic areas, but have occasionally been involved in the past in urban epidemic episodes.

The following species of fleas serve as principal vectors of the infection: *Xenopsylla cheopis*, *Xenopsylla gerbilli minax*, *Xenopsylla gerbilli caspica*, *Xenopsylla skrjabini*, *Xenopsylla conformis*, *Citellophilus tesquorum*, *Neopsylla setosa*, *Oropsylla silantiewi*.

In addition to rodents, camels have been implicated in the causation of human plague.

As confirmed by most extensive investigations, involving the yearly examination of about one million rodents and five million fleas, during the last two years (1957-1958) plague has been altogether absent from the Pre-Caspian area and from Transbaikalia. The situation has remained serious in the Central Asiatic foci, where more than 4000 plague strains were isolated in 1957 and 1958, and to a lesser degree in the Transcaucasus area (isolation of about 600 strains). In spite of the high incidence of rodent infection, human plague was absent.

Plague studies in China have confirmed the existence of foci of the infection in (a) inner Mongolia and south-west Manchuria, where *Citellus dauricus* is considered as the reservoir of the infection, and (b) north Manchuria, where the reservoir is believed to be *Marmota sibirica*. In south China, the infection has been found to persist in commensal rodents (*R. norvegicus* and *R. rattus*) inhabiting the fields. In the north, *Meriones*, gerbils and *M. musculus* serve as facultative carriers of the infection, the last-mentioned species also fulfilling the same role in the south. The last serious outbreak, which occurred in 1944, was centred in the Tungliao district and neighbouring areas of south-west Manchuria. Then a gradual decline set in and plague is now no longer reported in the south. However, the infection still persists in the north, several hundred strains having been isolated as late as 1951. Systematic observations according to the pattern adopted in the Soviet Union have been started.

In the Mongolian People's Republic, plague is enzootic in the Siberian marmot (tarabagan), which inhabits an area of some 10 million hectares. This rodent is of great economic importance, being hunted for the sake of its fur, meat and fat. Plague outbreaks starting in persons hunting the animals or handling its fur, meat or fat are frequent and often assume a pneumonic character. This focus, which has remained fully active, is therefore potentially most dangerous and will prove difficult to control.

As a result of recent studies in the western part of the United States, emphasis has shifted from the larger wild rodents, such as the ground squirrels, to species such as *Microtus* and *Peromyscus*. On account of their small size and ecological inconspicuousness, insufficient attention has been paid to these species in the past. Recent investigations have confirmed that they are markedly resistant to plague infection. The two flea species prevalent on these small, free-living rodents, especially on *Microtus californicus*—thought to be the main plague reservoir in a recently investigated focus of California on account of its marked resistance to *P. pestis* and of its heavy infestation—are *Hystrichopsylla linsdalei* and *Malareaus telchinus*. The former has been found to be an efficient vector when blocked. The latter, apparently transmitting the infection mainly with its contaminated mouthparts, is apt to act as a vector of considerable secondary importance if attacking the rodents *en masse*. The possibility of a transfer of the wild rodent fleas to commensal rats has been experimentally demonstrated with radioactively tagged *M. telchinus*. These observations deserve close attention, in view of the possible co-existence of *Microtus* and *Peromyscus* with the commensal rats in the vicinity of human habitations.

Relationship between wild-rodent plague and domestic-rodent plague in different areas and conditions: peridomestic plague and its significance

It was considered that a classification of the numerous rodents implicated in the causation of plague under the three headings of commensal, semi-domestic and wild species is of limited value, inasmuch as many of the animals fall under different categories in different areas, or under two or all three categories in one and the same area. Nevertheless, it seemed as a rule advisable to retain this widely accepted system for all practical purposes. However, the experts were not unanimous on this point. One of them considered it more useful to distinguish between (a) rodents which have direct contact with man, and (b) rodents having no direct contact. In the opinion of another expert, it was not justified or correct to place in separate categories plague among domestic, peridomestic and wild rodents. Taking account of the peculiarities of the ecology of the rodents, the transfer of the plague bacillus among them, the laws governing the epidemiology of plague, and trends of further plague research, he considered it more correct to substitute for the above terminology the common term "rodent plague".

Variations in the virulence of *Pasteurella pestis* and in the susceptibility of domestic and wild rodents to plague infection

Virulence

After a long discussion, during which the value of the various methods recommended for the determination of the virulence of *P. pestis* was assessed, it was finally agreed that the problem should be approached in its ecological aspects rather than solely from the laboratory point of view.

It was admitted that the strains isolated from human plague victims were almost invariably of maximum virulence when tested on genetically-known susceptible laboratory animals. It was also maintained that variations in virulence, if present under natural conditions, did not appear to interfere with the perpetuation of plague.

Resistance of rodents to plague

Recent work on wild-rodent plague in Iran, Kenya, the Soviet Union, India, and Indonesia has shown that many rodents are refractory to plague. These findings are based on observations in the field and in the laboratory. It was formerly believed that markedly resistant rodents were of no importance in the epidemiology of plague, but it is now considered that such animals are a key factor in the perpetuation of the disease, which depends on an equilibrium between resistance and susceptibility to the infection in the rodents.

The existing evidence suggests that, although a passing acquired immunity is a universal phenomenon, inherent resistance is only a feature of certain species, such as the *Meriones* and *Rhombomys* in Iran and the Soviet Union, *Arvicanthus* in Kenya, *Microtus* and *Peromyscus* in California.

Vector efficiency in plague, with particular reference to survival of vectors under natural conditions

Vector efficiency

Further studies on the methods of evaluating the vector capacity of plague-infected fleas have been made, especially in the United States and the Soviet Union. It is recommended that studies on the capacity of fleas to transmit the infection be continued with these or with similar but improved methods. Nevertheless, the workers themselves expressed the view that their studies merely provide a guide to the interpretation of field observations. Indeed, such observations showed that the ability of fleas to transmit plague is of a complex nature and may vary among different strains of one and the same species.

Survival of vector fleas

Further observations have shown that under natural conditions many plague-infected fleas are able to survive for long periods, for instance, in a

starved condition for at least a year. Such field observations have been found useful in the Soviet Union in predicting manifestations of plague.

It may be claimed that the main role of plague-resistant rodents is to ensure the survival of plague-infected fleas.

THE PRESENT STATE OF THE THERAPY OF PLAGUE

The Committee admitted the great value of antibiotics in the treatment of severely affected plague patients, especially those suffering from pneumonic plague. At the same time, it was pointed out that quite satisfactory results could be obtained with sulfonamides in the treatment of bubonic plague and, if commenced early, occasionally even in pneumonic plague. Moreover, it was emphasized that in countries where only limited means and a limited staff were available, treatment with sulfonamides alone could be used on a large scale. Among the sulfonamides, sulfamerazine has maintained its superior position.

The Committee was informed that streptomycin, while highly efficient in the treatment of plague, was apt to produce highly resistant variants of *P. pestis*. The prompt use of other antibiotics may then save the patient. Experience has shown that the tetracycline group of antimicrobial drugs is as efficacious as streptomycin, produces fewer side-effects, and can be administered by the oral route. In the Soviet Union, mycerin and bacteriomyacin have proved satisfactory in the treatment of all types of plague.

The danger that overdoses of antimicrobial drugs may release toxin, should be constantly kept in mind. The simultaneous use of different antimicrobial drugs should be avoided.

The Committee took cognizance of the report that in the Soviet Union the use of unpurified horse or donkey antiplague serum had produced untoward reactions. However, the danger of complications in the form of serious toxæmia can probably be averted by using purified antitoxic sera, preferably rabbit gamma-globulin concentrates in combination with chemotherapy.

CONTROL

Recent progress in control measures against wild and commensal rodents

The Committee was informed of the results of large-scale and carefully planned antirodent campaigns in the Soviet Union. The aim of the operations varied in the different areas. In the Pre-Caspian focus, particularly on the right bank of the Volga, the work has been carried out with the intention of eradicating plague. To the south of the Volga—Ural sandy areas in western Kazakhstan—and in Chita province the object has been to *forestall* the possibility of the development of epizootics. In the other

areas, particularly in the Central Asian and Transcaucasian foci, work has been carried out with the object of *suppressing* intensive plague epizootics, and the creation of rodent-free belts was resorted to in order to reduce the prevalence of human plague.

In the Pre-Caspian area, gassing with chloropicrin and later with a hydrocyanic acid preparation were formerly used, but for the last seven or eight years distribution of grain treated with zinc phosphide from aeroplanes has been the method of choice. Poisoning with zinc phosphide was also the main method used against the gerbillinae in Central Asia. Barium fluoroacetate was found to be more efficient, but dangerous to other animals and even man. However, by analogy with previous experiences in China, poisoning in man was ascribed to lack of adequate supervision or to carelessness. To kill the marmots in the Transbaikalian focus, chloropicrin and cyanide preparations were used. It was pointed out that the difficulties of carrying out these large-scale campaigns were almost overwhelming and that an enormous staff had to be employed. The necessity of carrying out the work in the short period between the end of hibernation and the beginning of aestivation created further difficulties. The success of these operations, conducted yearly in an enormous area, is therefore all the more remarkable.

It was again emphasized that methods of biological control applied to rodents were ineffective and sometimes dangerous.

The methods recommended for the control of commensal rodents by the WHO Expert Committee on Insecticides are dealt with in Annex 3, page 34.

Recent progress in the control of plague vectors¹

With regard to recent statements that in certain areas fleas may have acquired a resistance to insecticides (see summary of the relevant reports in Annex 5, page 37), the Committee noted that up to the present no such resistance has been demonstrated in the species dangerous for the spread of plague, or at least it has not become manifest enough to be of practical importance. Nevertheless, the Committee noted with satisfaction that standard tests for determining resistance or susceptibility to insecticides will soon be available, and recommended that these should be systematically applied whenever insecticides were used for the purposes of plague control.

Experience in India has shown that residual spraying with DDT, as used on a large scale for malaria control, is also effective in reducing the number of fleas in the houses. As antimalaria operations will presumably be stopped soon, an increase of the fleas is likely to occur in

¹ See Annex 4, page 36.

consequence. In the opinion of the Committee, this should be brought to the attention of the health administrations concerned and suitable measures should be adopted.

In general, the experts insisted upon the necessity of using suitable insecticides at the proper time, with constant re-assessment of their efficiency and tests for the absence of resistance.¹

As it is generally agreed that the application of DDT in dust form is a most effective method against fleas, the Committee expressed great interest in the use of patch-dusting and particularly in the use of this procedure in conjunction with bait boxes.² This method may be preferable from the point of view of efficiency, as well as of economy.

Vaccination against plague

The knowledge concerning the state of immunity in man following plague infection is still incomplete. Consequently the members of the Committee felt that further intensive investigations are most necessary. However, it was again reported that vaccines, either living or killed, and containing adequate amounts of immunizing material, when administered in two doses, were capable of diminishing the susceptibility to infection. This is indicated by a definitely lower incidence of frank clinical plague in vaccinated persons. The most convincing evidence comes from Madagascar, where annually repeated vaccination has been largely responsible for a very low level of plague.

In the opinion of the Expert Committee, the principle of booster-dose administration is valuable, but not as an emergency measure. The Committee also recognizes that plague vaccination definitely potentiates the value of antimicrobial drugs, and it again reiterates the opinion expressed in the second report of the Expert Committee on Plague, which reads as follows:

“Annual revaccination with a single dose progressively improves the immune status of the population groups and thus serves as an effective means in the control of plague in endemic areas.”³

Experience in the USSR indicates that neither the EV strain nor the bivalent dry living vaccine “1.17”, used in obligatory vaccination programmes, was completely satisfactory. Extensive research has therefore been instituted to find a more effective vaccine. The Committee recommends,

¹ In this context, the definition of resistance is as follows: “Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species. The term ‘behaviouristic resistance’ describes the development of the ability to avoid a dose which would prove lethal.” (*Wld Hlth Org. techn. Rep. Ser.*, 1957, **125**, 7)

² See: Kartman, L. (1958) An insecticide-bait-box method for the control of sylvatic plague vectors, *J. Hyg. (Lond.)*, **56**, 455

³ *Wld Hlth Org. techn. Rep. Ser.*, 1953, **74**, 6

however, that while such investigations should be undertaken in the interested countries, vaccination against plague infection should be practised with the available preparations, either in two doses, if killed, or in one annual dose, if alive, or according to the principles which proved efficient in the hands of the local authorities.

Recommendations for the organization of epidemiological surveys on wild-rodent plague

The experts agreed that it was impossible to recommend an organization suitable for all foci of wild-rodent plague. In practice, attention is sometimes concentrated upon examination of the fleas alone, sometimes only the rodents are examined, and sometimes both methods are used in combination. The Committee was of the opinion that the combined method is the best. It is necessary to use a sufficiently large staff, well equipped with rapid means of transportation and adequate laboratory facilities. At the same time, it is essential to make arrangements by which small contingents of workers can be rapidly detached and sent to scenes of emergencies. Concise instructions for the work to be performed by the central staff and the detachments must be issued for each particular area.

The techniques for work in the field and in the laboratory have been described many times in different languages,¹ and have been supplemented by the method described by Mr. Frank M. Prince.²

Recommendations for the organization of plague control services

In the opinion of the experts, it is necessary to make provision in each plague area for a central antiplague service, one of the duties of which is to organize and to control the activities of the various peripheral institutions and detachments. The peripheral units must be under the direction of fully qualified medical officers, who must have at their disposal an adequate staff of specialists for the various branches of the work, as well as an ample supply of equipment, antimicrobial drugs and insecticides. However, where the situation requires it, stocks of antimicrobial drugs and insecticides may be left in the hands of the local officials in order to save as many lives as possible.

To prevent a situation developing such that the appearance of human plague is the first indication of the presence of the infection in an area, it is essential to maintain a constant watch for early manifestations of plague

¹ See, for example, Pollitzer, R. (1954) *Plague*, Geneva (*World Health Organization: Monograph Series*, No. 22); Baltazard, M., et al. (1956) *Bull. Wld Hlth Org.*, **14**, 457; Baltazard, M., et al. (1958) *Recherches sur la peste en Inde* (unpublished document WHO/Plague/46); and Tumansky, V.M. (1958) *Microbiology of plague—microbiologic bases of plague diagnosis*, 2nd ed., Moscow, Medgiz.

² See Annex 6, page 38.

in rodents and fleas. This enables control measures to be instituted at the earliest possible moment.

In order that these may be efficient as well as economical, no effort should be spared to delimit the affected areas as rapidly and as thoroughly as possible. However, it is essential to carry out control measures not only in the focus delimited, but in a sufficiently wide belt around it.

Prophylaxis of epidemics in endemic plague areas

The Committee reiterates the recommendations made in the second report of the Expert Committee on Plague regarding the value of prophylaxis with antimicrobial drugs for the contacts of pneumonic plague patients. It has, however, taken cognizance of the fact that in view of the comparatively high incidence of pneumonic plague in the USSR, the authorities have instituted chemoprophylaxis of all contacts, whether of bubonic or pneumonic plague, by treating them with 1 gram of streptomycin daily for a period of five days.

The use of seroprophylaxis is no longer recommended.

Possibilities for the suppression of plague in selected areas

It has been demonstrated that plague can be eradicated from extensive areas by a mass campaign against rodents and fleas, as has been reported from the Soviet Union. However, as this is not possible in every situation, it cannot be recommended as a general policy. At the same time, the eradication of the disease in selected areas is feasible, but whenever this is contemplated, the practicability of the project should be ascertained by a pilot study before the campaign is extended to the total area.

Training of personnel

In the opinion of the experts, routine training of the whole personnel, adapted to the local conditions, ought to be supplemented by granting special training facilities to staff members with a suitable background. As in the Soviet Union, such facilities, extending for a period of 4-6 months, ought to be granted not only to medical graduates, but also to other specialists, such as biologists (ecologists), parasitologists and zoologists.

In addition, selected staff members ought to be granted fellowships by the World Health Organization or other organizations to work for 6-12 months at least in antiplague institutes or organizations abroad.

Public health education

The programme adopted for public health education and propaganda ought also to be suited to the local conditions. Though it may be desirable to initiate the programme with the aid of specialists coming from

abroad, the actual work must be in the hands of locally recruited staffs. It may be desirable to undertake specific education of the public in the prevention of plague, either with the aid of health councils, formed by leaders of the community, or by specially trained demonstrators or small teams.

Instructions for medical practitioners and personnel of the medical services

In view of the deplorable fact that whenever plague becomes rare it is less often diagnosed in time, special efforts ought to be made to acquaint medical practitioners, nurses, midwives and other public health personnel likely to see plague patients with the features of the disease and its emergency treatment, pending hospitalization of the sufferers.

PROVISIONS OF THE INTERNATIONAL SANITARY REGULATIONS

When examining the plague provisions of the International Sanitary Regulations, the Committee noted that the major emphasis was placed on plague in ports, airports, and on board ship. It further noted that at the present time the danger of the spread of plague by international traffic was limited. However, in view of the possible extension of wild-rodent plague to urban and to port and airport areas, the Committee was of the opinion that, *except as indicated below, the provisions of the International Sanitary Regulations relating to plague should be maintained without change.*

In view of the fact that the risk of international transmission resulting from the presence of plague in wild rodents is very small and for practical purposes may be ignored, the Committee is of the opinion that a more realistic approach to international quarantine notification of infected and non-infected plague local areas would be to interpret the provisions of Articles 1 and 6 of the International Sanitary Regulations as follows:

Article 1: "*infected local area*" means—

...*(b)* a local area where plague infection among *rats* exists on land...

Article 6:

...*(c)* in the case of *rat* plague, one month has elapsed after suppression of the *plague infection*.

RECOMMENDATION FOR CO-ORDINATED RESEARCH

The Committee recommended that all possible attention be given to the following research programme:

1. Systematic research on wild-rodent plague in all endemic areas (ecology, dynamics of epizootics, ecological studies, interaction between rodents and fleas).

2. Studies on the variability of *P. pestis* under natural conditions and co-ordinated research on the virulence of *P. pestis*.
3. Studies on the pneumotropism of plague strains.
4. Methods of ascertaining the presence of the infection in the absence of manifest plague.
5. Studies on the relationship of *P. pseudotuberculosis* to *P. pestis* and on the natural occurrence of the former organism in rodents and man.
6. Systematic studies aimed at the production of more potent plague vaccines.
7. Studies on the biological control of vectors with special reference to their infection with microsporidia and other pathogenic protozoa.
8. Organization of reference laboratories.
9. Granting of facilities for mutual visits to research leaders in the key plague areas.
10. Exchange of information and plague reports and literature.

Discussing this programme, the experts emphasized that:

- (a) even though plague incidence has recently been low, the potential threat of this infection remains;
 - (b) the present low ebb of the incidence of the disease affords particularly good opportunities for studying the various plague problems.
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Annex 1

**RODENTS AND LAGOMORPHA OTHER THAN THE
COSMOPOLITAN SPECIES OF RATS
(*Rattus norvegicus* and *Rattus rattus* subsp.) AND OF COMMENSAL
MICE (*Mus musculus* subsp.) PROVED NATURALLY PLAGUE-
INFECTED OR STRONGLY INCRIMINATED THROUGH
POSITIVE FINDINGS IN THEIR ECTOPARASITES**

RODENTIA

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>
BATHYERGI- DAE	<i>Cryptomys</i> sp. White-toothed mole-rat	Angola
CAVIIDAE ¹ Caviinae	<i>Cavia aperea</i> Restless cavy	Brazil
	<i>Cavia pamparum</i> Pampas cavy or Lund's guinea-pig	Argentina
	<i>Cavia tschudii atahualpae</i> Peruvian cavy ²	Peru
	<i>Caviella australis australis</i>	Argentina
	<i>Caviella australis joannia</i>	Argentina
	<i>Galea musteloides leucoblephara</i>	Argentina
	<i>Galea musteloides littoralis</i>	Argentina
	<i>Galea spixii</i> "Preá"	Brazil ³
	<i>Kerodon rupestris</i> Brazilian rock-cavy	Brazil
CHINCHIL- LIDAE	<i>Lagostomus maximus immollis</i> "Vizcacha" or Peruvian hare	Argentina

¹ The common guinea-pig, *Cavia porcellus* (*Cavia cobaya* auctt.), though repeatedly found plague-infected, has not been included in this list since it is a domestic rather than a wild rodent.

² Some other subspecies of *Cavia tschudii* have also been found infected in Peru.

³ An unidentified species of *Galea* was also found naturally infected with plague in Bolivia.

Family and subfamily	Species	Locality
DIPODIDAE Dipodinae	<i>Allactaga elater</i> Small five-toed jerboa <i>Allactaga elater indica</i> <i>Allactaga major</i> (<i>A. jaculus</i> auctt.) <i>Allactaga sibirica saltator</i> <i>Allactaga sibirica sibirica</i> Mongolian five-toed jerboa (<i>A. saliens</i> auctt.) <i>Dipus sagitta</i> Northern three-toed jerboa <i>Eremodipus lichtensteini</i> <i>Pygeretmus platyurus</i> Greater flat-tailed jerboa <i>Scirtopoda telum</i> Thick-tailed three-toed jerboa	South-east Russia; Transcaucasia; Central Asia Iranian Kurdistan South-east Russia Transbaikalia South-east Russia Central Asia Transcaspia South-east Russia
ECHIMYIDAE Echimyinae	<i>Cercomys cunicularius laurentius</i> <i>Cercomys inermis</i>	Brazil Brazil
GEOMYIDAE	<i>Thomomys bottae</i> Western pocket-gopher <i>Thomomys fossor</i> (<i>Thomomys talpoides fossor</i> auctt.) Mountain pocket-gopher	California, USA; Colorado, USA (?) Fleas only: Colorado, USA
HETERO- MYIDAE Dipodominae Heteromyinae	<i>Dipodomys</i> sp. <i>Dipodomys ordi ordi</i> Ord's kangaroo-rat <i>Perognathus parvus</i> Pocket-mouse <i>Heteromys anomalus anomalus</i>	Texas, USA Washington, USA Fleas only: Washington, USA Venezuela
MURIDAE Cricetinae	<i>Akodon dolores</i> <i>Akodon mollis mollis</i>	Argentina Peru-Ecuador border region

Family and subfamily	Species	Locality
MURIDAE Cricetinae (contd)	<i>Akodon mollis orophilus</i> Mountain field-mouse	Huancabamba, Peru
	<i>Cricetulus barabensis</i>	Transbaikalia, Manchuria
	<i>Cricetulus eversmanni</i>	Mongolia
	<i>Cricetulus migratorius</i> Migratory (grey) hamster	South-east Russia
	<i>Cricetus cricetus</i> Common hamster	South-east Russia
	<i>Eligmodontia hirtipes jucunda</i>	Argentina
	<i>Eligmodontia moreni</i>	Argentina
	<i>Graomys griseoflavus centralis</i>	Argentina
	<i>Graomys griseoflavus griseoflavus</i>	Argentina
	<i>Hesperomys bimaculatus</i>	Argentina
	<i>Hesperomys fecundus</i>	Bolivia
	<i>Hesperomys laucha</i>	Argentina
	<i>Hesperomys murillus cordovensis</i>	Argentina
	<i>Hesperomys venustus</i>	Argentina; Bolivia
	<i>Holochilus balnearum</i>	Argentina
	<i>Holochilus sciureus</i> Sugar-cane rat	Brazil
	<i>Mystromys albicaudatus</i> White-tailed rat	South Africa
	<i>Neotoma albigula albigula</i> White-throated wood-rat	Arizona, USA; New Mexico, USA
	<i>Neotoma cinerea occidentalis</i> Western bushy-tailed wood-rat	California, USA
	<i>Neotoma desertorum</i> Desert wood-rat	Nevada, USA; Utah, USA
<i>Neotoma fuscipes</i> Dusky-footed wood-rat	California, USA; fleas only: Oregon, USA	
<i>Neotoma fuscipes mohavensis</i> Mohave desert wood-rat	Nevada, USA	
<i>Neotoma intermedia intermedia</i> (<i>N. lepida intermedia</i> auctt.) Intermediate (Rhoads') wood-rat	California, USA	
<i>Neotoma lepida lepida</i>	Utah, USA (?)	

Family and subfamily	Species	Locality
MURIDAE Cricetinae (contd)	<i>Neotoma micropus</i> Pack-rat	Texas, USA ¹
	<i>Onychomys</i> sp.	Fleas only: Texas, USA
	<i>Onychomys leucogaster</i> White-bellied grasshopper mouse	Fleas only: New Mexico and other western areas of the USA
	<i>Onychomys torridus</i>	Fleas only: New Mexico, USA
	<i>Oryzomys andinus</i>	Peru
	<i>Oryzomys arenalis</i>	Peru
	<i>Oryzomys flavescens</i> subsp.	Argentina; Bolivia
	<i>Oryzomys laticeps intermedius</i>	Brazil
	<i>Oryzomys laticeps nitidus</i>	Fleas only: Ecuador
	<i>Oryzomys palustris</i> (<i>Hesperomys palustris</i> auctt.)	New Orleans, Louisiana, USA
	<i>Oryzomys phaeopus olivinus</i>	Ecuador
	<i>Oryzomys pyrrhorinus</i>	Brazil
	<i>Oryzomys stolzmanni stolzmanni</i> (<i>O. longicaudatus stolzmanni</i> auctt.)	Huancabamba, Peru
	<i>Oryzomys xantheolus xantheolus</i>	Ecuador; Peru ²
	<i>Oxymycterus paramensis</i>	Bolivia
	<i>Peromyscus boylii</i> Brush-mouse	Fleas only: Arizona, USA
	<i>Peromyscus leucopus</i> White-footed mouse	Fleas only: New Mexico, USA
	<i>Peromyscus maniculatus</i> Deer-mouse	New Mexico, USA; fleas only: California, USA; Washington, USA
	<i>Peromyscus truei gilberti</i> Gilbert's white-footed mouse	California, USA

¹ Plague in *Neotoma* sp. was recorded in Oklahoma as well as in other western parts of the USA.

² An unidentified subspecies of *Oryzomys xantheolus* (? *O. x. baroni*) was also found infected.

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>	
MURIDAE Cricetinae (contd)	<i>Peromyscus truei truei</i> True's white-footed mouse	California, USA; New Mexico, USA ¹	
	<i>Phyllotis amicus amicus</i>	Peru	
	<i>Phyllotis amicus maritimus</i>	Fleas only: Peru	
	<i>Phyllotis darwini yaccarum</i>	Argentina	
	<i>Phyllotis fruticicolus</i>	Ecuador	
	<i>Phyllotis wolffsohni</i>	Bolivia	
	<i>Reithrodontomys megalotis</i> Harvest mouse	Fleas only: California, USA; Kansas, USA; New Mexico, USA	
	<i>Rhipidomys equatoris</i>	Peru	
	<i>Rhipidomys leucodactylus</i>	Bolivia	
	<i>Sigmodon hirsutus</i>	Venezuela	
	<i>Sigmodon hispidus</i> Cotton-rat	Fleas and lice only: New Mexico, USA	
	<i>Sigmodon peruanus</i>	Peru	
	Dendromyinae	<i>Dendromus haymani</i>	Belgian Congo
		<i>Dendromus mesomelas kivu</i>	Belgian Congo
		<i>Malacothrix typicus</i> Mouse-gerbil	South Africa
<i>Steatomys pratensis</i> Fat mouse		South Africa	
Gerbillinae	<i>Desmodillus auricularis</i> Namaqua gerbil	South Africa	
	<i>Gerbillus paeba</i>	South Africa	
	<i>Meriones libycus erythrourus</i>	Iranian Kurdistan; Transcaucasia	
	<i>Meriones meridianus</i> (<i>Plassiomys meridianus</i> auctt.) Midday gerbil	South-east Russia; Transcaspia; Turkestan	
	<i>Meriones persicus persicus</i>	Iranian Kurdistan	
	<i>Meriones tamariscinus</i> Tamarisk gerbil	South-east Russia	

¹ The presence of plague in *Peromyscus* sp. has also been repeatedly recorded in the western parts of the USA.

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>	
MURIDAE Gerbillinae (contd)	<i>Meriones tristrami</i>	Iranian Kurdistan	
	<i>Meriones unguiculatus</i>	Mongolia	
	<i>Meriones vinogradovi</i>	Iranian Kurdistan	
	<i>Rhombomys opimus</i> Great gerbil	South-east Russia; Central Asia	
	<i>Tatera brantsi</i> (<i>T. lobengulae</i> auctt.)	South Africa	
	<i>Tatera indica</i> Indian gerbil or antelope-rat	India	
	<i>Tatera schinzi</i> Schinz's gerbil	South Africa	
	<i>Tatera valida beniensis</i>	Belgian Congo	
	Microtinae	<i>Alticola worthingtoni semicanus</i> (<i>A. semicanus</i> auctt.)	Mongolia
		<i>Arvicola terrestris</i>	South-east Russia
<i>Ellobius lutescens</i>		Iranian Kurdistan	
<i>Ellobius talpinus</i> Northern mole-vole		South-east Russia	
<i>Lagurus curtatus</i> Sage-brush vole		Fleas only: Washington, USA	
<i>Lagurus lagurus</i> Steppe lemming		South-east Russia	
<i>Microtus arvalis</i> Common vole		South-east Russia	
<i>Microtus (Lasiopodomys) brandti</i> Brandt's vole		Transbaikalia, Mongolia	
<i>Microtus californicus</i> Californian meadow-mouse		California, USA ¹	
<i>Microtus (Stenocranius) gregalis raddei</i> Narrow-skulled vole		Transbaikalia	
<i>Microtus montanus</i> Mountain vole		Oregon, USA; fleas only: Washington, USA	
<i>Microtus nanus</i>		Fleas only: Washington, USA	
<i>Microtus socialis</i> Social vole		South-east Russia	

¹ Plague in *Microtus* sp. has also been recorded on several occasions in the western part of the USA.

Family and subfamily	Species	Locality
MURIDAE Microtinae (contd)	<i>Microtus townsendi townsendi</i>	Tacoma, Washington, USA
Murinae	<i>Acomys cahirinus</i> Cairo spiny-mouse	Egypt
	<i>Aethomys kaiseri medicatus</i>	Belgian Congo
	<i>Apodemus agrarius</i>	China
	<i>Apodemus sylvaticus</i> Common field-mouse	Transcaucasia
	<i>Arvicanthis niloticus</i> subsp.	Kenya
	<i>Arvicanthis niloticus niloticus</i> Nile-rat	Egypt
	<i>Arvicanthis niloticus nubilans</i> Unstriped African grass-rat	East Africa
	<i>Arvicanthis niloticus rossi</i>	Belgian Congo
	<i>Arvicanthis niloticus rufinus</i>	Senegal
	<i>Bandicota bengalensis</i> (<i>Gunomys bengalensis</i> auctt.) Lesser bandicoot-rat	Burma; India
	<i>Bandicota bengalensis kok</i> (<i>Gunomys kok</i> auctt.)	India
	<i>Bandicota gracilis</i>	Ceylon
	<i>Bandicota indica</i> Large bandicoot-rat	India
	<i>Bandicota indica</i> (<i>Bandicota malabarica</i> auctt.)	Ceylon
	<i>Cricetomys gambianus</i> Giant rat	Belgian Congo; Ghana; Senegal
	<i>Dasymys incommutatus bentleyae</i> ¹ Swamp-rat	Belgian Congo
	<i>Grammomys dolichurus</i>	East Africa
	<i>Grammomys dryas</i>	Belgian Congo

¹ It has been stated that "*D. i. nudipes* [*Dasymys incommutatus nudipes*] is found in the enzootic plague area in Barotseland, N. Rhodesia, where it is subject to secondary infection from gerbils and *Mastomys*". [Davis, D.H.S. (1950) In: Union of South Africa, Department of Health, Plague Research Laboratory, *Sylvatic plague in South Africa: reservoirs and vectors*, Johannesburg (Special Report No. 1/50)]

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>	
MURIDAE Murinae (contd)	<i>Lemniscomys griselda</i> ¹	Senegal	
	<i>Lemniscomys striatus massaicus</i>	East Africa	
	<i>Lemniscomys striatus striatus</i> Spot-striped grass-mouse	Belgian Congo	
	<i>Lophuromys aquilus</i> (<i>L. aquilus rita</i> auctt.)	Belgian Congo	
	<i>Millardia meltada</i> Soft-furred field-rat or metad	India	
	<i>Mus booduga</i> (<i>Leggada booduga</i> auctt.) Little Indian field-mouse	India	
	<i>Mus deserti</i> (<i>Leggada deserti</i> auctt.) Dwarf-mouse	South Africa	
	<i>Mus musculoides emesi</i> (<i>Leggada emesi</i> auctt.) Pygmy-mouse	Belgian Congo	
	<i>Mus triton fors</i> (<i>Leggada triton fors</i> auctt.)	Belgian Congo	
	<i>Mylomys cunninghami alberti</i> (<i>M. dybovskii alberti</i> auctt.)	Belgian Congo	
	<i>Pelomys fallax iridescens</i> ²	East Africa	
	<i>Rattus natalensis</i> (<i>Mastomys</i> or <i>Rattus coucha</i> auctt.) Multimammate mouse	South Africa; Belgian Congo; Kenya	
	<i>Rhabdomys pumilio</i> Four-striped grass-mouse	South Africa; Kenya	
	Otomyinae	<i>Otomys</i> sp.	East Africa
		<i>Otomys angoniensis</i> Swamp-rat	Kenya
		<i>Otomys irroratus</i> South African water-rat	South Africa
		<i>Otomys tropicalis elgonis</i>	Belgian Congo
		<i>Otomys unisulcatus</i> (<i>Myotomys unisulcatus</i> auctt.)	South Africa
		<i>Parotomys brantsi luteolus</i> Eastern Karroo rat or Brants' otomys	South Africa

¹ This rodent was originally described as *Pelomys campanae* [Garnham, P. C. C. (1949) *Bull. Wld Hlth Org.*, **2**, 271] but was later identified as *Lemniscomys griselda* (*Bull. Wld Hlth Org.*, 1951, **3**, 697).

² Davis (1950) noted that "in Barotseland [Northern Rhodesia] *P. f. frater* [*Pelomys fallax frater*] is associated with *Otomys* and *Dasyomys* and has a similar flea fauna. It may act as a transient reservoir with these species." [Davis, D.H.S. (1950) In: Union of South Africa, Department of Health, Plague Research Laboratory, *Sylvatic plague in South Africa: reservoirs and vectors*, Johannesburg (Special Report No. 1/50)].

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>
PEDETIDAE	<i>Pedetes capensis</i> South African spring-hare	South Africa
SCIURIDAE	<i>Citellus armatus</i> Uinta ground-squirrel	Western part of the USA (Idaho, Nevada, Montana, Wyoming, Utah, Washington)
	<i>Citellus beecheyi beecheyi</i> California ground-squirrel	California, USA
	<i>Citellus beecheyi douglasi</i> Douglas ground-squirrel	California, USA; Oregon, USA
	<i>Citellus beecheyi fisheri</i> Fisher's ground-squirrel	California, USA
	<i>Citellus beecheyi nudipes</i>	Fleas only: California, USA
	<i>Citellus beldingi beldingi</i> Belding's ground-squirrel	California, USA
	<i>Citellus beldingi oregonus</i> Oregon ground-squirrel	California, USA; Nevada, USA; Oregon, USA
	<i>Citellus columbianus columbianus</i> Columbian ground-squirrel	Washington, USA
	<i>Citellus columbianus ruficaudus</i> Blue Mountain ground-squirrel	Oregon, USA
	<i>Citellus dauricus dauricus</i> Dauria sisek	Transbaikalia
	<i>Citellus erythrognys pallidicauda</i>	Mongolia
	<i>Citellus fulvus</i> Large-toothed suslik	South-east Russia
	<i>Citellus idahoensis</i> Idaho ground-squirrel	Fleas only: Idaho, USA
	<i>Citellus lateralis chrysodeirus</i> Golden-mantled ground-squirrel	California, USA
	<i>Citellus lateralis lateralis</i> (<i>Callospermophilus lateralis</i> auctt.)	Fleas only: Wyoming, USA ¹
	<i>Citellus leucurus leucurus</i> (<i>Amnospermophilus leucurus</i> auctt.) Antelope ground-squirrel	Fleas only: Arizona, USA; California, USA

¹ Plague was also confirmed in fleas from *Callospermophilus* sp. in California and in fleas and ticks from *Citellus lateralis* in Colorado.

Family and subfamily	Species	Locality
SCIURIDAE (contd)	<i>Citellus major</i> Red-cheeked suslik	Western Kazakhstan
	<i>Citellus mexicanus</i>	Fleas only: New Mexico, USA
	<i>Citellus pygmaeus</i> Little suslik	South-east Russia
	<i>Citellus richardsoni elegans</i> Wyoming ground-squirrel	Wyoming, USA
	<i>Citellus richardsoni nevadensis</i> Nevada ground-squirrel	Nevada, USA
	<i>Citellus richardsoni richardsoni</i> Richardson's ground-squirrel	Alberta and Saskatchewan, Canada; Montana, USA
	<i>Citellus spilosoma major</i> Spotted ground-squirrel	Fleas only: New Mexico, USA
	<i>Citellus townsendi mollis</i> Piute ground-squirrel	Fleas only: Idaho, USA
	<i>Citellus tridecemlineatus</i> 13-striped ground-squirrel	Fleas only: New Mexico, USA; Texas, USA
	<i>Citellus undulatus</i> (<i>C. rufescens</i>)	Mongolia
	<i>Citellus variegatus grammurus</i> Say's rock-squirrel	Utah, USA; fleas only: Arizona, USA; Colorado, USA; New Mexico, USA
	<i>Citellus variegatus utah</i> Utah rock-squirrel	Utah, USA
	<i>Citellus washingtoni loringi</i> Loring's ground-squirrel	Washington, USA
	<i>Citellus washingtoni washingtoni</i> Washington ground-squirrel	Washington, USA
	<i>Cynomys</i> sp.	Fleas only: Colorado, USA; Texas, USA
	<i>Cynomys gunnisoni gunnisoni</i> Gunnison's prairie-dog	New Mexico, USA
	<i>Cynomys gunnisoni zuniensis</i> Zuni prairie-dog	Arizona, USA; New Mexico, USA

Family and subfamily	Species	Locality
SCIURIDAE (contd)	<i>Cynomys leucurus</i> White-tailed prairie-dog	Fleas and lice only: Wyoming, USA
	<i>Cynomys ludovicianus</i> Black-tailed prairie-dog	USA: Colorado, Kansas, Montana, New Mexico, Texas, Wyoming
	<i>Cynomys mexicanus</i>	North Mexico
	<i>Cynomys parvidens</i> Utah prairie-dog	Utah, USA
	<i>Funambulus</i> sp. (? <i>F. pennanti</i>)	South India
	<i>Funambulus palmarum</i> Indian palm-squirrel	Ceylon; India
	<i>Glaucomys sabrinus lascivus</i> Sierra Nevada flying-squirrel	California, USA
	<i>Marmota baibacina centralis</i> (<i>Arctomys centralis</i> auctt.)	Russian Turkestan
	<i>Marmota caudata</i> Long-tailed marmot	Central Asia
	<i>Marmota flaviventris</i> subsp. Yellow-bellied marmot	Colorado and Oregon, USA; Fleas: British Columbia, Canada; New Mexico, USA
	<i>Marmota flaviventris avara</i>	Fleas only: Oregon, USA
	<i>Marmota flaviventris engelhardti</i> Engelhardt's marmot	Montana, USA; Utah, USA; Wyoming, USA
	<i>Marmota flaviventris nosophora</i> Golden-mantled marmot	Montana, USA
	<i>Marmota sibirica</i> Siberian marmot or tarabagan	Manchuria; Mongolia; Transbaikalia
	<i>Sciurus stramineus nebouxi</i> Neboux's squirrel	Ecuador; Peru
	<i>Spermophilopsis leptodactylus</i> Long-clawed ground-squirrel	Central Asia
	<i>Tamias minimus</i> (<i>Eutamias minimus</i> auctt.) Least chipmunk	Fleas only: Washington, USA

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>
SCIURIDAE (contd)	<i>Tamias quadrivittatus frater</i> (<i>Eutamias speciosus frater</i> auctt.) Tahoe chipmunk <i>Tamiasciurus douglasi albolimbatus</i> Sierra Nevada chickaree <i>Xerus erythropus</i> Central African side-striped squirrel <i>Xerus inauris</i> (<i>Geosciurus capensis</i> auctt.) Bristly ground-squirrel	California, USA; Nevada, USA California, USA Senegal South Africa

LAGOMORPHA

<i>Family and subfamily</i>	<i>Species</i>	<i>Locality</i>
LEPORIDAE	<i>Lepus californicus</i> Black-tailed jack rabbit	California, USA
	<i>Lepus capensis</i> Cape hare	South Africa
	<i>Lepus europaeus</i> European hare	England; Argentina
	<i>Lepus saxatilis</i> Scrub hare	South Africa
	<i>Lepus tolai</i>	Central Asia
	<i>Oryctolagus cuniculus</i> ¹ Rabbit	England
	<i>Sylvilagus</i> sp. Cotton-tail rabbit	Bolivia; Ecuador; Peru
	<i>Sylvilagus andinus</i>	Huancabamba, Peru
	<i>Sylvilagus audoboni</i> Desert cotton-tail rabbit	New Mexico, USA
	<i>Sylvilagus bachmani</i> California brush-rabbit	Fleas only (?): California, USA
	<i>Sylvilagus brasiliensis</i>	Brazil
	<i>Sylvilagus brasiliensis gibsoni</i>	Argentina; Bolivia
	<i>Sylvilagus caudatus</i> "Tapeti"	Huancabamba, Peru
<i>Sylvilagus nuttalli nuttalli</i> Washington cotton-tail rabbit	California, USA	
OCHOTONIDAE	<i>Ochotona daurica</i>	Transbaikalia
	<i>Ochotona pricei</i>	Mongolia

¹ Instances of secondary plague manifestations among domesticated rabbits, due to the presence of the infection among rats, have been recorded by several observers.

Annex 2

**FLEAS OF RODENTS OTHER THAN THE COSMOPOLITAN
SPECIES OF RATS (*R. rattus* and *R. norvegicus* subsp.)
AND OF COMMENSAL MICE (*M. musculus*) FOUND TO
BE NATURALLY INFECTED WITH *P. pestis***

<i>Species</i>	<i>Locality</i>	<i>Usual hosts</i>
<i>Adoratopsylla</i> (<i>Tritopsylla</i>) <i>intermedia coph</i> a	Ecuador	<i>Didelphis</i>
<i>Amphipsylla primaris mitis</i>	Mongolia	—
<i>Anomiopsyllus</i> sp.	New Mexico, USA	<i>Neotoma</i>
<i>Anomiopsyllus hiemalis</i>	Texas, USA	<i>Neotoma</i>
<i>Atyphloceras</i> sp.	Western part of USA	<i>Lagurus, Peromyscus</i>
<i>Atyphloceras multidentatus</i>	Western part of USA	<i>Peromyscus</i>
<i>Catallagia decipiens</i>	Washington, USA	<i>Lagurus, Peromyscus</i>
<i>Cediopsylla spillmanni</i>	Huancabamba, Peru	<i>Sylvilagus</i>
<i>Chiastopsylla rossi</i>	South Africa	<i>Tatera, Otomys</i> and other wild rodents
<i>Citellophilus lebedewi</i>	Central Asia	<i>Marmota</i>
<i>Citellophilus tesquorum</i> subsp.	South-east Russia; Transbaikalia	<i>Citellus</i>
<i>Coptopsylla bairamaliensis</i>	Central Asia	<i>Rhombomys</i>
<i>Coptopsylla lamellifer ardua</i> (<i>Coptopsylla lamellifer fallax</i> auctt.)	Central Asia	<i>Meriones, Rhombomys</i>
<i>Coptopsylla lamellifer dubinini</i>	Central Asia	<i>Meriones, Pallasiomys, Rhombomys</i>
<i>Coptopsylla lamellifer rostrata</i>	Transcaspia	<i>Meriones, Rhombomys</i>
<i>Ctenophthalmus breviatus</i>	South-east Russia	<i>Citellus, Microtus</i>
<i>Ctenophthalmus cabirus</i>	Belgian Congo	<i>Arvicanthis</i> and other rodents
<i>Ctenophthalmus dolichus</i>	Central Asia	<i>Meriones, Pallasiomys, Rhombomys</i>
<i>Ctenophthalmus phyris</i>	Belgian Congo	<i>Arvicanthis, Lemniscomys, Otomys</i>
<i>Ctenophthalmus pollex</i>	South-east Russia	<i>Arvicola, Citellus</i>

<i>Species</i>	<i>Locality</i>	<i>Usual hosts</i>
<i>Ctenophthalmus secundus</i>	Transcaucasia	<i>Microtus</i>
<i>Delostichus (Parapsyllus) talis</i>	Argentina	<i>Cavia</i>
<i>Diamanus montanus</i>	Western part of USA	<i>Citellus</i>
<i>Dinopsyllus ellobius</i> (<i>D. lypusus</i> auctt. nec J. & R.)	South Africa	<i>Rhabdomys, Tatera</i> and other rodents
<i>Dinopsyllus lypusus</i>	East Africa; Belgian Congo	<i>Arvicanthis</i> and other wild rodents
<i>Echidnophaga oschanini</i>	Transcaspia; Central Asia; Mongolia	<i>Rhombomys</i>
<i>Foxella ignota</i>	Colorado, USA	<i>Thomomys</i>
<i>Frontopsylla semura</i>	South-east Russia	<i>Citellus</i>
<i>Hectopsylla eskeyi</i>	Peru	<i>Cavia</i> and rats
<i>Hectopsylla suarezi</i>	Ecuador	<i>Cavia</i> and rats
<i>Hoplopsyllus andensis</i>	Huancabamba, Peru	<i>Sylvilagus</i>
<i>Hoplopsyllus anomalus</i>	Western part of USA	<i>Citellus</i>
<i>Hoplopsyllus glacialis affinis</i> (<i>H. affinis</i> auctt.)	New Mexico, USA	<i>Sylvilagus</i>
<i>Hoplopsyllus manconis</i> (<i>H. exoticus</i> auctt.)	Huancabamba, Peru	<i>Sylvilagus</i>
<i>Hystrichopsylla linsdalei</i>	California, USA	<i>Microtus</i>
<i>Listropsylla dorippae</i>	South Africa	<i>Tatera</i>
<i>Malaraeus telchinus</i>	Western part of USA	<i>Microtus, Peromyscus</i>
<i>Megabothris clantoni</i> <i>clantoni</i>	Washington, USA	<i>Lagurus, Peromyscus</i>
<i>Meringis shannoni</i>	Washington, USA	<i>Lagurus</i> and other wild rodents
<i>Mesopsylla apscheronica</i>	Central Asia	<i>Allactaga</i>
<i>Mesopsylla eucta tuschkan</i>	Central Asia	<i>Alactagulus, Allactaga,</i> <i>Scirtopoda</i>
<i>Monopsyllus eumolpi</i>	Western part of USA	<i>Tamias</i>
<i>Monopsyllus exilis</i>	Texas, USA	<i>Onychomys</i>
<i>Monopsyllus wagneri</i>	Western part of USA	<i>Lagurus, Peromyscus</i> and other wild rodents

<i>Species</i>	<i>Locality</i>	<i>Usual hosts</i>
<i>Neopsylla mana</i>	Mongolia	—
<i>Neopsylla setosa</i>	South-east Russia	<i>Citellus</i> and other wild rodents
<i>Neotyphloceras rosenbergi</i>	Ecuador	<i>Didelphis</i> and wild rodents
<i>Nosopsyllus</i> sp.	Iranian Kurdistan	<i>Meriones</i>
<i>Nosopsyllus (Gerbillophilus) aralis</i>	Central Asia	<i>Meriones</i>
<i>Nosopsyllus consimilis</i>	South-east Russia	Mice
<i>Nosopsyllus laeviceps</i>	South-east Russia	<i>Lagurus</i>
<i>Nosopsyllus mokrzeckyi</i>	South-east Russia	Mice
<i>Nosopsyllus (Gerbillophilus) tersus</i>	Central Asia	<i>Rhombomys</i>
<i>Nosopsyllus (Gerbillophilus) turkmenicus</i>	Central Asia	<i>Meriones, Pallasiomys, Rhombomys</i>
<i>Odontopsyllus</i> sp.	Huancabamba, Peru	<i>Sylvilagus</i>
<i>Ophthalmopsylla volgensis</i>	South-east Russia; Central Asia	<i>Alactagulus, Allactaga, Dipus</i>
<i>Opisocrostis hirsutus</i>	Western part of USA	<i>Cynomys</i>
<i>Opisodasys keeni nesiotus</i>	California, USA	<i>Microtus, Peromyscus, Reithrodontomys</i>
<i>Orchopeas leucopus</i>	New Mexico, USA	<i>Peromyscus</i>
<i>Orchopeas neotomae</i>	New Mexico, USA	<i>Neotoma</i>
<i>Orchopeas sexdentatus</i>	Western part of USA	<i>Neotoma, Peromyscus</i>
<i>Oropsylla ilovaiskii</i>	South-east Russia	<i>Citellus</i>
<i>Oropsylla silantiewi</i>	Manchuria; Mongolia; Transbaikalia	<i>Marmota</i>
<i>Paradoxopsyllus dashidorzhii</i>	Mongolia	—
<i>Paradoxopsyllus teretifrons</i>	Central Asia	<i>Meriones, Pallasiomys, Rhombomys</i>
<i>Peromyscopsylla hesperomys adelpha</i>	New Mexico, USA	<i>Peromyscus</i>
<i>Pleochaetis dolens quitanus</i>	Huancabamba, Peru	<i>Cavia, Oryzomys, Sylvilagus</i>
<i>Pleochaetis equatoris</i>	Huancabamba, Peru	<i>Akodon, Oryzomys, Sylvilagus</i>

<i>Species</i>	<i>Locality</i>	<i>Usual hosts</i>
<i>Plocopsylla hector</i>	Ecuador	<i>Thomasomys</i> and other wild rodents
<i>Polygenis</i> sp.	Ecuador Raquia, Peru Venezuela	<i>Akodon</i> <i>Oryzomys</i> <i>Heteromys</i> , <i>Sigmodon</i> , rats
<i>Polygenis brachinus</i>	Huancabamba, Peru	<i>Akodon</i> , <i>Oryzomys</i>
<i>Polygenis litargus</i>	Ecuador-Peru border region Huancabamba, Peru	<i>Oryzomys</i> , <i>Sciurus</i> <i>Akodon</i> , <i>Oryzomys</i>
<i>Polygenis platensis cisandinus</i>	Argentina	<i>Cavia</i> and other rodents
<i>Rhadinopsylla cedestis</i>	Transcaucasia	<i>Meriones</i> , <i>Pallasiomys</i> , <i>Rhombomys</i>
<i>Rhadinopsylla ukrainica</i>	South-east Russia	<i>Microtus</i>
<i>Rhadinopsylla ventricosa</i>	Central Asia	<i>Marmota</i>
<i>Sphinctopsylla mars</i>	Huancabamba, Peru	<i>Hesperomys</i>
<i>Stenistomera (Miochaeta) macrodactyla</i>	New Mexico, USA	<i>Peromyscus</i>
<i>Stenoponia conspecta</i>	Central Asia	<i>Rhombomys</i>
<i>Stenoponia insperata</i>	Iranian Kurdistan; Transcaucasia	<i>Meriones</i>
<i>Stenoponia vlasovi</i>	Central Asia	<i>Meriones</i> , <i>Pallasiomys</i>
<i>Thrassis bacchi bacchi</i> (= <i>Thr. gladiolis</i>)	Western part of USA	<i>Citellus</i>
<i>Thrassis bacchi johnsoni</i>	Western part of USA	<i>Lagurus</i> , <i>Peromyscus</i>
<i>Thrassis fotus</i>	Western part of USA	<i>Onychomys</i>
<i>Thrassis stanfordi</i>	Western part of USA	<i>Marmota</i>
<i>Tiamastus cavicola</i> (<i>Rhopalopsyllus cavicola</i> auctt.)	Ecuador; Peru	<i>Cavia</i>
<i>Xenopsylla buxtoni</i>	Iranian Kurdistan	<i>Meriones</i>
<i>Xenopsylla conformis</i>	Central Asia; Transcaspia	<i>Meriones</i>
<i>Xenopsylla eridos</i> (<i>X. pasiphae</i>)	South Africa	<i>Otomys</i> and other wild rodents
<i>Xenopsylla gerbilli caspica</i>	Central Asia; Transcaspia	<i>Meriones</i> , <i>Rhombomys</i>

<i>Species</i>	<i>Locality</i>	<i>Usual hosts</i>
<i>Xenopsylla gerbilli minax</i>	Central Asia; Transcaspia	<i>Meriones, Rhombomys</i>
<i>Xenopsylla hirtipes</i>	Central Asia ; Transcaspia	<i>Allactaga, Meriones, Rhombomys</i>
<i>Xenopsylla nuttalli</i>	Transcaspia	<i>Rhombomys</i>
<i>Xenopsylla philoxera</i> (<i>Xenopsylla eridos</i> auctt.)	South Africa	<i>Tatera</i> and other wild rodents
<i>Xenopsylla phyllomae</i>	South Africa	<i>Desmodillus</i> and other wild rodents
<i>Xenopsylla piriei</i>	South Africa	<i>Desmodillus</i>
<i>Xenopsylla skrjabini</i>	Central Asia; Transcaspia	<i>Rhombomys</i>
<i>Xenopsylla versuta</i>	South-west Africa	<i>Rhabdomys</i>
<i>Xiphopsylla lippa</i>	Belgian Congo	<i>Lophuromys</i> and other wild rodents

Annex 3

**METHODS RECOMMENDED
FOR THE CONTROL OF COMMENSAL RODENTS ***

The importance of sanitation, including proper garbage disposal, food storage, harbourage elimination, and rat-proofing, must be emphasized. Sanitation is essential to the permanent control of commensal rats and mice, and the use of rodenticides should be regarded as supplementary to sanitation.

The situation with regard to rodent control is quite encouraging, since rats, unlike insects, do not appear to have developed resistance to the available poisons. The slow-acting rodenticides—warfarin, Pival, coumachlor, and fumarin—are preferred for use in most situations because of their effectiveness and low degree of toxic hazard to humans and useful animals.

Warfarin

It has been shown that the susceptibility to warfarin of the various species of commensal rodents differs. Accordingly, in the interest of economy and safety, the lowest bait concentration consistent with the most effective control should be used.

Dependable control of the roof rat, *Rattus rattus*, requires the use of a concentration of 0.250 mg of warfarin per gram of bait (250 p.p.m.). Field tests have shown that a concentration of 0.050 mg per gram (50 p.p.m.) is effective for control of the Norway rat (*R. norvegicus*). Mice react in the same general way as Norway rats, although they show more individual variation. There is evidence that under certain conditions Norway rats may be controlled a little more rapidly, though not more surely, by using 0.100 mg of warfarin per gram of bait (100 p.p.m.) instead of 0.050 mg. There is a real opportunity for those associated with municipal programmes to determine, by careful operational observations, which of these two concentrations is the more desirable for use in organized control campaigns. However, where the species of rat concerned is not definitely known, the 0.25-mg/g concentration should be used. Commercial preparations generally contain 0.5 % warfarin in corn starch. To obtain the 0.25-mg/g concentration 1 part (by weight) of concentrate is mixed with 19 parts of bait.

Warfarin baits may be used for initial rodent control under practically all conditions, using a minimum baiting period of two weeks. Consideration should be given to establishing permanent bait stations for control of rats in places which are subject to re-infestation. Two years of experience in

* Extract from eighth report of the Expert Committee on Insecticides (*Wld Hlth Org. techn. Rep. Ser.*, 1958, 153, 44).

one country have shown that Norway rats could be controlled in non-rat-proof buildings so long as poisoned bait is available, but eradication was not achieved. No difference in the residual effectiveness of the two bait concentrations (0.100 and 0.050 mg/g) was observed. The bait stations were inspected approximately every six weeks and fresh poisoned bait was supplied.

Pival

Pival (2-pivalyl-1,3-indandione) appears, in a general way, to be approximately equal to warfarin in its rodenticidal effectiveness. In both laboratory tests and simulated field trials, the acceptance of Pival baits by roof rats, Norway rats, and mice has been good. Laboratory tests showed that Pival and warfarin were about equally good for mouse control. Against roof rats, Pival was shown to be slightly better than warfarin in the laboratory and about the same under simulated field conditions. Both laboratory tests and simulated field studies indicated that Pival in solid bait was somewhat inferior to warfarin for control of the Norway rat, but that Pival-poisoned water (0.006% acid equivalent) with 5% sugar as an attractant was slightly more effective against this species than a comparable formulation of warfarin (0.005% acid equivalent). Pival concentrations in solid baits should not be less than 0.025%.

Corn meal, as well as some of the other ground grains, has proved to be a good bait material for use with rodenticides that require repeated acceptance over several days. However, in many areas the meal becomes insect-infested after several days of exposure and must be replaced at frequent intervals with fresh bait in order to assure continued acceptance by rodents. Pival has been found to inhibit the development of a number of insects that infest grain, including flour beetles (*Tribolium* spp.), cigarette beetles (*Lasioderma serricornis* [F.]), saw-toothed grain beetles (*Oryzaephilus surinamensis* [L.]) and flour moths (*Ephestia* spp.). Cockroaches, however, were not affected when fed for 30 days on bait containing 0.25 mg of Pival per gram of bait.

ANTU

ANTU still holds a definite place as a quick-acting poison for the Norway rat. Its use to reduce large populations rapidly may be followed by the use of anticoagulant rodenticides to achieve complete and lasting control. ANTU should not be used against the same populations more often than about once a year. It induces a persistent bait shyness in rats, and this property makes ANTU ineffective for repeated use against the same rat population. ANTU is not effective for the control of roof rats or mice. Its safety record is good, and it may be used in residences and food-handling establishments. The concentration generally employed in solid baits is 2-3%.

Sodium monofluoroacetate (1080)

This rodenticide is still the most effective, fast-acting rodenticide, but its extreme toxicity to man and animals requires that it be used exclusively for certain types of premises and only by carefully trained crews. The precautions necessary for the safe use of sodium monofluoroacetate are numerous and involved.

Baiting problems

In general, any available cereal may be used as bait material. It is worth while to re-state that under certain conditions it is very difficult to get rodents to take any bait—e.g., where food is available in great variety and abundance, as in some warehouses. Such conditions constitute “baiting problems,” a term which implies that the origin of the difficulty lies not in the particular poison used but in the ecology of the rodents themselves. Obviously, in testing any given rodenticide formulation, it is necessary to be certain that apparent failure of the formulation is not caused by baiting problems. These problems can be solved effectively only through extensive knowledge of the habits of the rodents. Such problems do not occur more frequently with simple cereal baits such as corn meal than with complex bait mixtures. However, where the meal becomes insect-infested or mouldy after several days of exposure, it should be replaced.

Baiting for rodents needs to be carried out with fresh materials of the best possible quality. Frequent replacement of (not addition to) baits is very important, regardless of the presence of insects, moulds, or other factors which may cause contamination. It is often worth while to extend the baiting to the environs of properties being protected. This is most important around non-rat-proofed buildings used for food storage.

Annex 4**METHODS FOR THE CONTROL OF PLAGUE VECTORS ****Oriental rat flea*

Xenopsylla cheopis, the principal transmitter of plague and endemic or murine typhus, is readily controlled by the application of a 5-10 % DDT dust to rat-runs and harbourage areas. While the 5 % dust is equal to the 10 % formulation in its effectiveness on *X. cheopis*, the greater toxicity of

* Extract from eighth report of the Expert Committee on Insecticides (*Wld Hlth Org. techn. Rep. Ser.*, 1958, 153, 41).

the latter to other ectoparasites normally less susceptible to DDT, such as the cat flea, makes it the formulation of choice. Although DDT has been used extensively against this species, there is little evidence to indicate any loss of control through the development of resistance.

In India, there is evidence that indoor residual spraying with DDT at a dosage of 2 g/m² (200 mg per square foot) has brought about a marked reduction in flea density. In the same country, carefully controlled field trials indicate that "patch dusting" is an easy, cheap and effective method of controlling *X. cheopis*. The dusting-powder is deposited in small quantities under grain bins, on rat-runs and in other places in infested premises, where it is not likely to be disturbed. Those employing the technique require little technical training. A programme may be successfully carried out by delivering the necessary amount of dusting-powder to each householder for distribution by him according to instructions. A check is later made by the programme supervisor to ensure that the work has been correctly performed. 1.5 % dieldrin and 2 % aldrin dusting-powders gave zero flea indices four months after application. An index of 0.3 was obtained with a 10 % DDT powder.

Repellents

Diethyltoluamide is a superior flea repellent, particularly when used to impregnate socks and outer garments... Clothing impregnated with diethyltoluamide repels fleas for more than one week. Temporary protection can be obtained by smearing the repellent on the socks and trouser legs. Undecylenic (or undecanoic) acid, propylacetanilide, and benzyl benzoate are also good flea repellents; clothing treated with them remains protected through several days of ordinary wear.

Annex 5

PRESENT EXTENT OF INSECTICIDE-RESISTANCE IN FLEAS

Xenopsylla cheopis

The oriental rat flea has been reported to be involved as an uncommon species in the control failures with DDT in Ecuador.¹ Indefinite indications of DDT-resistance insufficient to affect the control programme were reported by Good in 1951.² But by 1956 no effective resistance to DDT was shown by *X. cheopis* anywhere in its range in the United States (Hess, personal communication, 1956).

¹ Sáenz Vera, C. (1953) *Bull. Wld Hlth Org.*, **9**, 615

² Cited by Hess, A. D. (1952) *Amer. J. trop. Med. Hyg.*, **1**, 371

Two attempts have been made to increase the DDT-tolerance of *X. cheopis* by selection pressure in the laboratory. A strain at Savannah increased in tolerance for the first three generations, the mortality rate to a given dose of DDT decreasing from 65% to 33%, but it did not decrease further in subsequent generations.¹ The LC_{50} of DDT for a strain in London doubled in four generations.²

Annex 6

ORGANIZATION AND TECHNIQUES FOR EPIDEMIOLOGICAL SURVEYS ON WILD-RODENT PLAGUE

The necessity for epidemiological surveys on wild-rodent plague in specific areas is usually the result of: (1) reported human cases; (2) epizootics; (3) the finding of wild rodents, domestic rodents, and humans in close association, as in recreational areas or in the outskirts of cities and villages. To conduct surveys it is important that personnel be trained in public relations as well as in such specialized fields as mammalogy, entomology, and the pathology of plague in rodents. Contact with public health and local officials for their co-operation and assistance is of primary importance before actual survey operations begin. Publicity is quite often a problem if not properly handled, and it is necessary to furnish officials with factual and objective information for press releases to avoid hysteria among the local population. Once the officials and others concerned are familiar with the problem and background information is available, general observations can be made to determine the extent of the problem and the public health hazards which may be involved.

The surveys are best conducted with two trained operators but may be carried out efficiently with one trained man assisted by local personnel. In making general observations, trapping and hunting areas are selected on the basis of rodent activity in close proximity to human habitations and populations. Maps and general descriptions of the area are prepared and specific trapping sites are designated. General observations will usually give the experienced operator a good idea of the types of mammals in the area, and this knowledge will in turn determine the kind of trapping or hunting to be done.

In trapping small rodents (chipmunks and smaller), excellent results are obtained with "Museum Special" traps to obtain dead animals, and "Sherman" and "Young" traps for live specimens. For the smaller species of

¹ Kilpatrick, J. W. & Fay, R. W. (1952) *J. econ. Ent.*, **45**, 284

² Shawarby, A. A. (1953) *Bull. ent. Res.*, **44**, 377

ground squirrels, the "Official" rat trap is used, while the larger ground squirrels, marmots, and prairie dogs are shot with 20-gauge shot-guns or 0.22 calibre rifles. Although it is realized that a certain number of fleas leave the animals trapped by the dead-fall method, a high percentage of the trapped animals have one or more fleas when examined. The dead-fall trap is preferred to the live trap in epidemiological surveys because it enables a larger number of animals and a greater variety of species to be collected in a larger area per unit of time. Consequently, a more representative sample of flea species is obtained without any actual reduction in the absolute number available for laboratory testing, the loss of some fleas from dead animals being compensated by the fact that a larger number of hosts is taken than would be the case in live trapping. Fifty to 100 traps may be carried conveniently in a shoulder-strap canvas bag. Oatmeal, moistened with water to a sticky consistency, is used for baiting the traps. After baiting, the traps are placed 25 to 50 feet apart in locations most suitable for catching the different species of rodents in the area and marked by tying a piece of white cloth (about 2 in. wide and 12 in. long) to branches, wire fences, or other objects near the trap. With small nocturnal rodents, the best results are obtained by setting the traps in the late afternoon and attending them in the early morning. Late setting of traps prevents grasshoppers or other animal life from tripping them before animals have had an opportunity to be caught, and early morning attendance finds the animal in fresh condition with more fleas present.

Each host specimen is removed by carefully placing the animal and trap inside a bag before releasing the spring. Rough handling often jars the fleas from the specimens. Small animals are placed in paper bags, the tops of which are folded or twisted and then fastened securely. The bags are then properly labelled in pencil or non-smear ink with the specimen number, date, location, species, and other pertinent information. Animals are conveniently carried in the shoulder-strap canvas bag used for traps. When trapping operations are completed for a specific area, field-data sheets (specimen attached) are then filled out for the various species of animals collected. Animals from the same specific locality are numbered consecutively and kept in separate bags or other containers. Several animals of the same species may be all recorded on one data sheet with their respective specimen numbers. Fleas are either removed or left on the dead hosts. If they are removed, they are placed in vials containing 2% saline solution and associated with their host by number on the appropriate data sheet.

The field operations from this point on depend on the availability of an effective refrigerant and of rapid transportation to the laboratory. When dry ice (frozen CO_2) can be obtained without too much difficulty and when regularly scheduled air-express shipments can be made, the animals, with completed data sheets attached, are placed immediately after return from

the field into well insulated cardboard boxes containing the dry ice, and shipped to the laboratory periodically. If dry ice is not available, animals are examined for ectoparasites and autopsied in the field.

Shipping frozen specimens to the laboratory offers the following advantages over field examination: (1) more time is available for field observations and collecting; (2) field personnel do not need to take along instruments and other laboratory supplies; (3) the specimens can be thoroughly processed in a well-equipped laboratory by personnel trained in mammalogy, entomology and bacteriology.

When frozen specimens are received in the laboratory they are allowed to thaw and then carefully identified as to genus and species. The ectoparasites are removed by holding the rodent over a white enamelled pan and striking it sharply with a three-cornered file. The fleas are identified, placed in individual tubes, triturated with a glass rod, and streaked on blood agar plates. The residue is inoculated into test animals (white mice or guinea-pigs) after pooling in lots of 10 fleas or less. Other ectoparasites (lice, mites, and ticks) are identified, triturated in mortars, and inoculated into test animals.

After the ectoparasites have been removed, each animal is autopsied and examined carefully for gross lesions suggestive of plague. If pathological symptoms are observed, all diseased organs (spleen, lung, liver, nodes) are removed and examined microscopically; a portion of each organ is then inoculated subcutaneously into a test animal and another portion streaked on a blood agar plate. If there are no symptoms of plague, a portion of the spleen is removed and treated in the above manner, or pools are made from the spleens of several animals and inoculated subcutaneously into test animals.

In areas where it is impossible to secure dry ice and rapid transportation of specimens to the laboratory, animals must be examined in the field. The bags containing the animals are placed in a can, two or three tablespoonsful of cyanogas (calcium cyanide dust containing 42% active ingredients) are added, and the can is covered tightly; between 20 and 30 minutes are allowed to kill all ectoparasites. The cover is removed and the contents emptied and allowed to air for a few minutes before handling. The ectoparasites are removed from the rodents, as described above, and placed in No. 3 vials containing 2% saline solution for mailing to the laboratory. Fleas, lice, mites and ticks from each animal are put in separate vials, or the different groups of ectoparasites from the same species of rodent from the same location may be pooled. Ticks are best shipped alive in dry vials. The animals are autopsied and examined for symptoms of plague. Any suspicious tissues are removed and kept under refrigeration for shipment to the laboratory by the most rapid method available. If feasible, spleens are removed from animals of normal appearance, and pools of them are shipped to the laboratory for examination.

When shipments are received in the laboratory, the fleas and other ectoparasites are poured from the vials into Petri dishes, identified, and inoculated subcutaneously into test animals. Fleas of the same species from the same host and location are pooled; other ectoparasites are pooled and inoculated without separating into species. In all cases, care must be taken to keep records which will associate ectoparasites with their hosts.

Tissues received from the field are placed in mortars, macerated, and one test animal is inoculated subcutaneously while one is vaccinated (scarification).

All inoculated animals surviving are held for a period of from 10 to 14 days, then sacrificed and examined macroscopically and microscopically for *P. pestis*.

Specimen field-data sheet *

Specimen No. Date
(Collected)

Hosts
Number, genus and species

Degree of infestation - Scarce . Many
(Reason, if scarce)

Shot..... Trapped..... Found dead..... Sick.....

Location
State County Ranch or farm

US or State highway - Distance and direction nearest town on map

Nature of terrain
Elevation - Vegetation - Cultivated - Desert - Timbered - Rocky - etc.

Epizootics in this area
History of rumors of sickness or disappearance of rodents - Sick or dead animals seen-Abandoned nests

Specimens forwarded herewith
Number vials of parasites

Remarks:
.....
.....
.....

* As used in the USA.

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