PAN AMERICAN HEALTH ORGANIZATION

ADVISORY COMMITTEE ON MEDICAL RESEARCH

FIRST MEETING

18-22 JUNE 1962
WASHINGTON, D.C.

PLAGUE IN THE AMERICAS

Ref: RES 1/13
12 JUNE 1962

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION
WASHINGTON, D.C.

PLAGUE IN THE AMERICAS

Table of Contents

PLAGUE IN THE AMERICAS *

SECTION A

INTRODUCTION

Since the birth of the Pan American Health Organization plague has been an important albeit diminishing problem of the member countries. The initial meeting of the Organization, then known as the International Sanitary Bureau, was the First International Sanitary Convention held in Washington D.C. from 2 to 4 December, 1902. One of the main concerns of the founders was: "The adoption of measures for the disposal of garbage and wastes to prevent the spread of bubonic plague and other diseases".

In the years following, the application of classical methods has driven plague into the endemic foci of today. While current control and containment measures have been more or less successful, it has become obvious that before further progress can be made against plague it will be necessary to undertake a thorough study of the nature of the disease in its present circumstances.

As a first step in a program to include the needed ecological studies, a thorough study and evaluation was begun of all information on plague in the Americas contained in the technical literature, official reports and other sources. Based on these data and observations to be made in the plague foci, there will be designed a series of ecological research studies.

This document contains a summation of the basic information on plague in Venezuela, Ecuador, Peru, and the United States. Similar information on other plague-involved countries will be rendered in a further document in the near future. Included also in this document are a listing of research needs, and preliminary outliness of two field research projects, i.e. Peru and Venezuela. In undertaking the exhaustive studies necessary and in the preparation of this document the Organization has had the capable services of Dr. Robert Pollitzer on assignment from Fordham University, and Dr. K. F. Meyer, Director Emeritus of the George Williams Hooper Foundation, University of California Medical Center, together with the secretariat services of Dr. A. N. Bica and Dr. E. C. Chamberlayne.

---

* Prepared for the first meeting of the PAHO Advisory Committee on Medical Research, 18-22 June 1962.

SECTION B

GENERAL REMARKS

As can be gathered from the adjoined tabulations, plague is at present manifest in the western part of the United States as well as in five South American countries, namely Bolivia, Brazil, Ecuador, Peru and Venezuela. Generally speaking, the ecology of the disease is of a uniform pattern, characteristic also of other countries with a vast hinterland like, for instance, South Africa, which had become plague-infected during the present pandemic: entering through sea-ports, the infection involved first the rat-populations in these urban areas and soon also in more or less distant cities and towns, the rat epizootics invariably leading to considerable epidemics, However, though often slowly, the infection inexorably spread to rural areas of the hinterland where owing to the presence of susceptible wild rodent species, it found conditions for its persistance comparable in principle to those in the ancient plague foci of Central Asia. Though in some of the affected South American countries or parts thereof the common rats are still involved in the manifestations of plague, in other foci these rodents have ceased to play a role or have been relegated to a secondary role, becoming but temporarily affected when the disease is rampant among the wild rodents and thus, like the house mice and, more often, the domesticated guinea-pigs serving merely as links in the chain of events leading to a transition of the infection from the wild rodent reservoir to man (which, however, may also be effected through direct contact with wild rodents or through their fleas).

Thus, as these brief general statements suffice to show, considerable variances in the plague situations in the individual countries, or even foci involved, are bound to exist, and these variances are further accentuated by the presence of different wild-rodent and wild-rodent flea species in the various affected localities. Therefore, in order to arrive at an adequate appreciation of the plague situations in the presently involved American countries, it is necessary to deal individually with each of them.

TABLE I

Plague incidence in the presently affected countries of America during the period 1946-1955

Country	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955
Argentina	10	4	12	-	-	1	2	-	-	-
Bolivia	13	?	3	3	22	10	55	-	9	45
Brazil	332	88	386	91	55	20	65	10	6	27
Ecuador	45	21	40	19	28	33	44	90	81	85*
Peru	126	173	73	116*	77*	73*	60*	163	75	8
USA	-	1	-	-	-	-	-	-	-	-
Venezuela	-	-	7	2	5	8	-	1	-	-

* Deaths only

TABLE II

Plague incidence in the Americas from 1956-1961

(Figures culled mainly from the WHO Weekly Epidemiological Record)

II.1 Argentina

Year	Plague incidence
1956	-
1957	-
1958	One plague case recorded in the Misiones Province (see "Reported cases of notifiable diseases in the Americas 1949-1958", PAHO/WHO publication No. 48 - 1960, p. 70)
1959	-
1960	-
1961	-

II.2 Bolivia

Year	Plague incidence
1956	Three cases recorded in the Santa Cruz Department, Valle Grande Province (see PAHO/WHO publication No. 48)
1957	-
1958	-
1959	-
1960	Small epidemic (12 cases) recorded at Pucar, Santa Cruz Department, Valle Grande Province
1961	No information

TABLE II
(Cont'ed)

II.3 Brazil

Year	States affected	Case incidence Per State	Yearly total
1956	Pernambuco	4	4
1957	Alagoas	5	
	Pernambuco	12	
	Bahia	20	37
1958	Bahia	25	25
1959	Bahia	16	16
1960	Bahia	13	
	Rio de Janeiro	2	
	Alagoas	13	28
1961	According to not yet final figures published in the 1961 and 1962 Weekly Epidemiological Record, plague was present in 1961 in the following states: Alagoas, Bahia, Ceará, Minas Gerais, Paraiba, Pernambuco and Rio de Janeiro		

II.4 Ecuador

Year	Localities affected Provinces	Cantons	Case incidence Per Canton	Per Province	Yearly total
1956	Tungurahua	Ambato	18	18	
	Manabí	Jipijapa	12	12	
	Chimborazo	-	-	7	
	Loja	-	-	43	80

TABLE II (Cont'ed)

II.4 Ecuador (Cont'ed)

Year	Localities affected		Case incidence		Yeerly total
	Provinces	Cantons	Per Canton	Per Province	
1957	Los Rios	Vinces	5	5	
	Chimborazo	Guano	12	12	
	Loja	-	-	62	79
1958	Chimborazo	Guano	7		
		Riobamba	1	8	
	Loja	Celica	9		
		Cariamanga	1		
		Calvas	2		
		Macará	1	13	21
1959	Chimborazo	Guano	11		
		Riobamba	7	18	
	Loja	Celica	4		
		Macará	1		
		Loja	7		
		Catacocha	1		
		Faltas	6	19	37

TABLE II (Cont'ed)

II.4 Ecuador (Cont'ed)

Year	Localities affected: Provinces	Cantons	Case incidence: Per Canton	Per Province	Yearly total
1960	Tungurahua	Pillaro	5	5	
	Chimborazo	Riobamba	8		
		Alausi	10		
		Guano	15	33	
	El Oro	Pinas	5	5	
	Loja	Celica	1		
		Paltas	7		
		Calcas	17		
		Macará	11	36	79
1961	According to preliminary figures published in the 1961 and 1962 volumes of the Weekly Epidemiological Record, plague was reported in 1961 in the provinces of Tungurahua (Ambato Canton), Manabí (Portoviejo and Rocafuerte Cantons), El Oro (Pinas and Zaruma Cantons), Chimborazo (Guano and Riobamba Cantons) and Loja (Celica, Paltas and Calvas Cantons). (Information available up to the middle of May, 1962 indicates that in this year plague continued to be present in the provinces of Manabí, El Oro, Chimborazo and Loja).				

II.5 Peru

Year	Localities affected: Department	Province	Case incidence: Per Province	Per Department	Yearly total
1956	Piura	Ayabaca	8		
		Huancabamba	16	24	24

TABLE II (Cont'ed)

II.5 Peru (Cont'ed)

Year	Localities affected Department	Province	Case incidence Per Province	Per Department	Yearly total
1957	Piura	Ayabaca	6		
		Huancabamba	11	17	
	Ancash	Huaraz	11		
		Recuay	3	14	31
1958	Piura	C. Villar	3		
		Sullana	6		
		Ayabaca	24	33	
	Lambayeque	Lambayeque	7	7	
	Cajamarca	Hualgayoc	4	4	
	Ancash	Huaraz	5	5	49*
1959	Piura	Ayabaca	14	14	
	Cajamarca	Hualgayoc	18	18	32
1960	Piura	Ayabaca	15		
		Huancabamba	112	127	
	Cajamarca	Hualgayoc	7	7	134
1961	According to the WHO Weekly Epidemiological Record for 1961 and 1962 plague continued to occur in the provinces of Huancabamba and Ayabaca in 1961. (The presence of the disease in the Huancabamba Province was again recorded early in 1962, when also the Jaén Province of the Cajamarca Department became involved)				

* As stated in the PAHO/WHO Scientific Publication No. 48 ("Reported cases of notifiable diseases in the Americas 1949-1958 Washington 1960, p.70) three plague cases were recorded in 1958 in the Tumbes Department, the case incidence in the above mentioned departments being Piura 31, Lambayeque 7, Cajamarca 3 and Ancash 5.

TABLE II
(Cont'ed)

II.6 United States

Year	Incidence per State	ANNUAL total
1956	California 1	1
1957	Texas 1	1
1958	-	-
1959	California 2	
	New Mexico 1	3
1960	New Mexico 2	2

Three cases were reported in New Mexico in 1962

II.7 Venezuela

Year	State	Localities involved: District	Municipality	Case Incidence	Yearly Total
1956	Aragua	Ricaurte	Tejerias	3	3
1957	-	-	-	-	-
1958	-	-	-	-	-
1959	-	-	-	-	-
1960	-	-	-	-	-[a]
1961	Aragua	Ricaurte	Tejerias	2	
			El Consejo	1	
			Tumero	3	6[b]

Remarks -.(a) The Weekly Epidemiological Record for 1960 (Vol. 35, 7:80) stated that evidence of plague in a wild rodent (Sigmodon hispidus hirsutus) was detected on January 26 in the locality last found affected by human plague in 1956.

(b) One case of "bubonic sylvatic plague" was recorded in 1962 in the Municipality of La Victoria, Ricaurte District of the Aragua State (Wkly. epid. Rec. 1962, 37 (8):93).

SECTION C

REVIEW AND EVALUATION OF THE PRESENT STATUS OF PLAGUE IN VENEZUELA AS OBTAINED FROM THE TECHNICAL LITERATURE AND OFFICIAL REPORTS

History and recent incidence of plague

As can be gathered from the article of Díaz, the statements which were summarized and supplemented by Pollitzer and recently confirmed by De la Barrera, plague appeared in 1908 in the port of La Guaira and soon spread from there to Caracas, where it continued to occur until 1919, leading to a total of 204 cases with 99 deaths.

Even before this 'urban' phase of the disease had come to an end, the infection had spread in 1910 to rural areas of Miranda State, in parts of which it continued to become manifest from time to time until 1951. An adjacent part of Aragua State, reached by the infection in 1939, has remained involved to date. As can be seen from a table inserted in Pollitzer's book, the total case incidence in Miranda State from 1910 to 1933 was 206. The recently available information on the plague manifestations in that state as well as in Aragua may be summarized as follows:

Miranda State

Year	Locality	Case incidence	Remarks
1950	La Palma	5	No details known
1951	Mostaza (Munic.of San Pedro)	8	According to De la Barrera five persons were attacked in Puerto Escondido during the last week of July in one house, where one month before dead rats had been found. In the nearby community of Puesto Topo de la Mina three plague cases were recorded in one and the same house on July 31, August 15 and August 30.

Aragua State

Year	Locality	Cases	Deaths	Remarks
1939	Ricaurte district, La Florida	11	8	As stated by Díaz, this outbreak took place in the Hacienda La Florida, where 4 plague-affected rats had been found and *X. cheopis* and *X. brasiliensis* were met with in most of the houses.

Year	Locality	Cases	Deaths	Remarks
1943	Ricaurte district:			
	a) Guayita, Tejerías Munic.	10	3	According to Díaz the first 3 attacks occurred in a house where 20 domesticated guinea-pigs had died, the next two in a neighboring house. The other five sufferers were obviously infected during a wake held in the latter house, which appeared to have been much infested by rats and fleas.
	b) Trapiche de Medio, El Consejo Munic.	7	2	These attacks took place one month after the just mentioned outbreak and appeared to be epidemiologically related to it.
1948	La Horqueta	7	3	As related by De la Barrera, 4 persons fell ill with plague in one house between May 23-24 and 3 more from May 27 to 30.
1949	Las Palomas, La Pepeña	2	1	No details known
1953	Ricaurte district, Capachal, Tejerías Munic.	1	?	No details known
1960	Ricaurte district, Tejerías Munic.			According to the WHO Wkly. Epid. Record 35 (1960) 7:80, evidence of plague was found on Jan. 26 within the confines of Tejerías Municipality in a Sigmodon hispidus hirsutus. This is undoubtedly the animal succumbed to 'super-acute' plague referred to by De la Barrera.
1961	Ricaurte district	3	?	As summarized in Vol. 36, No. 28, p.305 of the WHO Wkly. Epid. Record, 2 bubonic cases were reported in April in the Tejerías Municipality and one in June in the El Consejo Municipality, a dead rat having been found in the house of one of the sufferers.
	Marino district	3	?	According to the same source 3 bubonic cases were detected in June in the Tumero Municipality of the Marino district.

Year	Locality	Cases	Deaths	Remarks
1962	Ricaurte district	1	?	As reported in the Wkly. Epid. Record 37 (1962) 8:93, one patient suffering from "bubonic sylvatic plague" was found in La Victoria Municipality in February of the present year.

As far as reliance can be placed upon these partly not yet confirmed data, it would appear that within recent years the plague manifestations in Venezuela were restricted mostly to the Ricaurte district of Aragua State, where the area of the Tejerías Municipality seems to form a particularly persistent focus of the infection. Certainly one should be most cautious to use the absence of manifestations of the disease in man as a means to assert the absence of plague in an area, since -- as was actually the case in Venezuela -- the infection may remain latent in the wild rodents for prolonged periods. Nevertheless one might reasonably ask if not (a) the now involved area in that country is less extended than was stated by Díaz, who estimated that the territories affected in the Miranda and Aragua States measured about 1000 km^2 (386 sq. miles) or whether (b) within this area strongholds of the infection (called 'elementary' plague foci by some recent Soviet authors) do exist, where the infection is particularly apt to persist during the interepizootic periods and which, therefore, may form the starting point of epizootic waves. As will be discussed later in this report, the detection of such territorially limited, particularly active plague foci within an affected area is a matter of utmost importance.

Observations on rodents and other ecologically important animals

(1) 'Domestic' species

As stated by De la Barrera, whose carefully checked observations deserve most credence, Rattus norvegicus occurs within the presently affected Venezuelan plague areas only in scanty numbers inside the houses of Las Tejerías town, while R. rattus is amply met with throughout the involved territories. According to this observer, the habitat of R. rattus rattus seems to be confined to the houses and their immediate vicinity, but he admits that owing to the small number of these rodents among the trapped animals it was impossible to come to valid conclusions regarding their ecology.

To judge from the large number of their captures, R.r. alexandrinus and R.r. frugivorus are frequent in both the urban and rural houses, the former appearing to be more common in the Venezuelan plague area than the latter. Both were also caught within a radius of at least 500 meters round the houses, having thus opportunities to come in contact with wild rodents approaching the houses.

Mus musculus appears to be frequent in both the urban and rural habitations of the plague area as well as in their vicinity. As asserted by Díaz, this species has never been found plague-infected in Venezuela. It is noteworthy, however, that though according to De la Barrera these rodents are scantily flea-infested, besides Ctenocephalides felis wild rodent fleas (Polygenis bohlsi bohlsi) have been found on them.

Guinea pigs are kept in the rural houses of the Venezuelan plague areas, but to a lesser extent than is the case in Ecuador and Peru. Nevertheless, the above recorded observations on the 1943 outbreak in Guayita suggest that in Venezuela, as well as in these two countries, the domesticated guinea pigs may be instrumental in conveying plague from the rodents to man.

(2) Wild rodents

Referring in his reports to the wild rodents met with in the Venezuelan plague areas, De la Barrera furnishes the following data:

Species	Number of captures	Percentage
Sigmodon hispidus	1950	62.9
Proechimys guairae	337	10.8
Akodon venezuelensis	324	10.4
Heteromys anomalus	233	7.5
Neacomys spinosus	96	3.1
Oecomys bicolor	62	2.0
Rhipidomys venezuelae	46	1.4
Zygodontomys thomasi	26	0.8
Sigmomys alstoni	22	0.6

Remark - For reasons which are not clear, De la Barrera does not include in this list Sciurus granatensis (31 captures) and Dasyprocta rubratta rubratta (10 captures).

To judge from the short notes added by De la Barrera, Sigmodon hispidus, Akodon venezuelensis, Neacomys spinosus, Sciurus granatensis and particularly often Heteromys anomalus were found to enter the houses. It is important to note that this list includes the only two wild rodent species thus far found plague-infected in Venezuela -- Sigmodon hispidus and Heteromys anomalus.

It is possibly also significant that, in contrast to the other species enumerated in the list, Sigmomys alstoni, suspected to be a plague reservoir, was not found throughout the affected area but restricted to the vicinity of Las Tejerías. However, this species was also met with outside the plague area in lower locations with a higher temperature, i.e., an environment different from that in most parts of the affected region.

Dasiprocta rubratta seems to be the only one of the above mentioned rodents which is hunted. Though it has been found slightly infested with the common wild rodent fleas Polygenis bohlsi bohlsi and P. klagesi samuelis, so far it has been neither plague-infected nor implicated in the causation of human affections.

(3) Lagomorpha

The lagomorpha enumerated by De la Barrera are Sylvilagus brasiliensis (12 captures) and Cuniculus paca venezuelensis (4 captures) which are both hunted. The former, because known to have been plague-infected elsewhere in South America and found in Venezuela to harbor Polygenis bohlsi bohlsi, certainly deserves attention as a possible plague reservoir.

(4) Marsupialia

Didelphis marsupialis marsupialis (26 captures), an animal of nocturnal habits, because much in contact with rodents and, on the other hand, apt to stay round the houses or even to enter them, seems rather suspect as a liaison animal, capable of bringing infected fleas to the environments of the domestic or domesticated rodents and of man. Actually, De la Barrera noted the presence of Polygenis species, including P. bohlsi bohlsi and P. klagesi samuelis, on these animals. It further deserves attention that allied species have been found plague-infected in other parts of South America.

Observations on fleas

(1) Rat fleas

The findings on rat fleas recently made by De la Barrera in the Venezuelan plague area stand in a most remarkable contrast to those recorded by earlier observers. Díaz (1948) stated in this connection that "the rats which frequent the houses, especially in the urban centers, were mainly infested with X. cheopis and X. brasiliensis. The rats of the sylvatic type (i.e. apparently the wild rodents) are mainly infested with Polygenis bohlsi, on rare occasions with X. brasiliensis.- The fleas index is generally low in the rural zone, in contrast to the high indices in the towns and settlements. Among the sylvatic rodents, the Arditta común (Gerlinguetus aestuans) has been found infested with X. cheopis."

Dealing with the most common fleas in Venezuela, Cova García and Tallaferro (1959) recorded, unfortunately without indicating where they had made their investigations, that they had found X. cheopis, not only on the common rats but also on Sigmodon hispidus hirsutus and Didelphis marsupialis marsupialis. On the other hand, they found Polygenis bohlsi bohlsi, or both R. norvegicus and R. rattus, and recorded the presence of

several species of wild rodent fleas, including Polygenis klagesi samuelis and P. roberti beebei on the latter rat.

De la Barrera (1960) recorded the following data on the fleas he had found to infest R. r. alexandrinus and R. r. frugivorus:

	Percentages of the fleas found on:			
	R. r. alexandrinus		R. r. frugivorus	
Flea species	In the houses	On the fields	In the houses	On the fields
P. klagesi samuelis	5.5	7.6	9.4	-
P. bohlsi bohlsi	38.8	38.4	32.0	46.6
P. roberti beebei	18.5	38.4	26.6	26.6
P. dunni	16.6	7.6	19.0	26.6
P. peronis	3.7	-	1.9	-
P. occidentalis steganus	3.7	-	3.8	-
Ct. felis felis	12.9	7.6	7.6	-
Totals	99.7	99.6	100.3	99.8
Total fleas examined:	54	13	53	15

Though the numbers of fleas examined, especially those on the rats trapped in the open was too small to give an exact idea of the frequency of the different species, De la Barrera's findings leave no room for doubt that X. cheopis was absent, the common rats in the Venezuelan plague area thus being presently infested almost exclusively by wild rodent fleas, among which P. bohlsi bohlsi, P. roberti beebei and, to a somewhat lesser extent, P. dunni, appeared to be most frequent.

Commenting upon these findings, De la Barrera stated that since X. cheopis "existed previously and the local climatic conditions have not become modified, it is logical to attribute the fact (of the disappearance of this flea) to the treatment with insecticides effected by the Plague Prevention Service. During the whole time of our work there were but very few fleas (Pulex irritans, Ct. felis, wild rodent fleas, etc.) in the houses. However, the action of the disinsectants does not seem to have been so efficacious against these fleas than against X. cheopis. Besides the possibility of a higher sensitivity of the latter, it is necessary to record that this species is essentially domestic and probably breeds only in this environment... If so, it is natural that the disinsectization can better reach it."

(2) Wild rodent fleas

Referring to the fleas of wild rodents and evidently also of lagomorpha collected under his direction, De la Barrera furnished the following global figures:

Species	Number collected	Percentage
Polygenis bohlsi bohlsi	3 099	50.4
P. klagesi samuelis	1 672	27.2
P. roberti beebei	491	8.0
P. dunni	207	3.3
P. occidentalis steganus	65	1.0
P. peronis	48	0.7
Rhopalopsyllus australis australis	475	7.7
Rh. cacicus	80	1.3
Rh. lugubris cryctogenes	2	0.03
Totals	6 139	99.63

As will be gathered from the adjoined table (see pg.15a), the number of fleas collected from the specimens of the individual wild-rodent species varied considerably, data of statistical significance having been obtained only in the case of some of the species. It appears to be certain, however, that species belonging to the genus Polygenis which, as has been shown above, also preponderate most markedly on the common rats, form the overwhelming majority of the wild-rodent fleas in the Venezuelan plague area. There can be also hardly any doubt that the vectors of the infection there will be found among the Polygenis fleas, allied species of which have been found definitely capable of conveying plague in Argentina, Ecuador and in Brazil. It remains to be seen, however, which species are implicated in Venezuela.

Observations on human plague

Dealing with the epidemiology of plague in the presently affected Venezuelan areas, De la Barrera emphasized that (1) though instances of infections in the fields had been observed, as a rule man contracted the disease within the houses, and (2) contrary to what was usually observed in bubonic plague outbreaks, multiple infections in one and the same house were frequent. Though he postulated that in Venezuela, as well as in South America in general, the common rats had become resistant to plague, he was inclined nevertheless to the belief that as a rule their temporary involvement, due to a transition of the infection from the wild rodents, was responsible for the intradomestically acquired human attacks. He further maintained that the appearance of multiple plague attacks in one and the same house could take place only if a highly efficient vector-flea was available there. Since in his opinion the wild-rodent fleas did not fall into this category, he came to the conclusion that in the plague outbreaks in Venezuela recorded by him, X. cheopis was still responsible for the intradomiciliary conveyance of the infection.

Evaluating these postulations, one must state that at first glance the apparently lessened incidence of plague observed in Venezuela within recent years, when X. cheopis seemed to be absent from the affected

NUMBERS AND PERCENTAGES OF FLEAS ON THE MAMMALIA SPECIES TABULATED BELOW

Fleas	Sigmodon hispidus	Heteromys anomalus	Akodon venezuel.	Dasyprocta rubratta	Neacomys spinosus	Proechimys guairae	Proechim cayennen.	Phipidomys venezuelae	Sciurus granatensis	Sigmomys alstoni	Zygo-dontomys thomasi	Sylvil brasi-liensis	Didelphis marsupialis
Polygemis bohlsi	2518/87.4	14/8.4	372/90.7	2/0.5	3/8.3	118/6.5	32/50.0	5/9.4	-	7/33.3	11/91.6	4/26.6	24/2.1
P. klagesi samuelis	121/4.1	9/5.4	7/1.7	16/4.7	2/5.5	1493/84.2	11/17.1	15/28.3	-	-	-	-	29/2.5
P. roberti beebei	127/4.4	11/6.6	12/2.9	-	31/86.1	106/5.9	-	32/60.3	1/10.0	4/19.0	1/8.3	-	152/13.4
P. dunni	79/2.7	84/50.9	12/2.9	-		18/1.0	-	-	1/10.0	10/47.6	-	-	3/0.2
P. peronis	3/0.1	42/25.4	-	-		-	-	-	-	-	-	-	1/0.08
P. occidentalis steganus	3/0.1	-	2/0.4	1/0.2		9/0.5	-	-	8/80.0	-	-	-	28/2.4
Rhopalopsyllus australis	1/0.03	-	-	313/92.0		2/0.1	-	1/0.9	-	-	-	10/66.6	5/0.4
Rh. cacicus	1/0.03	5/3.0	-	6/1.7		7/0.3	-	-	-	-	-	-	59/5.2
Rh. lugubris cryptoctenes	-	-	-	2/0.5		-	-	-	-	-	-	-	-
Adoratopsylla antiquorum	3/0.1	-	-	-		-	21/32.8	-	-	-	-	-	1/0.08
A. intermedia oxyura	2/0.06	-	-	-		10/0.5	-	-	-	-	-	-	804/70.8
Ctenocephalides felis	23/0.8	-	5/1.2	-		9/0.5	-	-	-	-	-	1/6.6	26/2.2

areas, seems to speak in favor of De la Barrera's views. Since, however, no doubt in the past this flea was considerably more numerous in the house than the present vectors of the infection (wild-rodent fleas) are now, the lessened incidence of plague and the absence of multiple attacks may be due merely to a scarcity of the vectors and not to their lessened capacity to convey the infection. It would not seem wise, therefore, to consider the Venezuelan wild-rodent fleas as inadequate plague vectors, before proof for this contention has been obtained through appropriate tests.

Summary and recommendations

The recent observations on plague in Venezuela, briefly dealt with in the foregoing pages, permit to conclude that:

(1) the infection has remained restricted for over two decades to adjacent districts of the Miranda and Aragua States, the focus evidently showing no tendency to expand;

(2) possibly at present plague is active only in a limited part of Aragua State which, as has been discussed above, may thus form a permanent stronghold of the infection;

(3) there can be no doubt that wild rodents, two species of which have been found definitely implicated, form the permanent plague reservoir;

(4) the 'domestic' rodent fauna, including besides the common rats and house mice in part also domesticated guinea pigs, becomes only temporarily involved in the wild rodent epizootics, an event apt to lead to the appearance of plague in man;

(5) the houses of the plague area having apparently been freed from X. cheopis, wild rodent fleas now form the usual, if not the sole, vehicle of the infection not only from rodent to rodent but also from the rodents to man.

Valuable as this information is, it must be realized that it furnishes only a general picture of the present plague situation in Venezuela, leaving many essential questions concerning the ecology of the disease unanswered. The most important among the problems of the latter category are that:

(a) the contention that plague is now restricted to a limited part of its original focus in the Miranda and Aragua States rests only on deductions made from the known incidence of human plague and must, therefore, be confirmed or disproved through wholesale and thorough examinations of the rodents and their fleas within the maximal limits of the affected area;

(b) the hitherto obtained knowledge on the presence of wild rodent plague is rather scanty, as it rests only on the apparently rather occasional detection of the infection in two of the several wild rodent species (and lagomorpha) met with in the affected areas. It is to be expected that the wholesale examinations proposed above will yield further information in this respect, but in addition to ample field investigations of as many rodent species as possible, it is also essential to test their susceptibility to plague in the laboratory. Standard methods must be used for the latter work so as to detect differences in the plague susceptibility of the various rodent species;

(c) to render the above mentioned field investigations effective and to pave the way for measures of controlling the wild rodents, they must be accompanied, or even preceded, by a thorough study of the ecology of the animals on which, however, presumably much local information is available;

(d) hand in hand with these field and laboratory studies of the wild rodents, thorough parallel studies must be made on the common rats, particularly in order to determine their susceptibility to plague. It has to be stressed in this connection that, though it has been often postulated that the rural rat populations, not only in Venezuela but in other wild-rodent plague foci of South America, have become resistant to the infection, thus far, the present reporters have failed to find any exact data proving this point;

(e) as has been noted already, it is still unknown which of the numerous wild-rodent flea (or, one might safely say, polygenis) species are involved in the spread of plague in Venezuela. Again, it may be expected that the field investigations will furnish information on this point, provided that care is taken to use exclusively lots consisting of only one well identified flea species for the cultivation of P. pestis and for pooling tests in guinea pigs. However, in addition to this, exact studies have to be made to determine the vector efficiency of the various species of wild rodent fleas. It would be advisable not only to use X. cheopis as a standard of comparison in these tests but also to study the vector efficiency of the local P. irritans and Ct. felis strains;

(f) in the course of the above field investigations it is also important to make thorough epidemiological and laboratory studies of all human plague cases observed at the time. If possible, the plague strains isolated from man as well as from naturally infected rodents and fleas ought to be kept in a lyophilized state for further studies;

(g) the question whether a campaign against the wild rodents would be a practical possibility can be decided only after the above outlined investigations have been well advanced, particularly after the presently plague-affected area has been adequately delimited;

(h) it ought to be clear that a detailed program for the above proposed investigations and studies can be drawn up only after consultation with the medical officers of the Venezuelan Plague Prevention Service and a preliminary survey in order to establish the manner in which the contemplated work could be conducted.

REFERENCES:

1) R. I. Díaz (1948) Aspectos epidemiológicos de la peste en Venezuela. Arch. venezolanos de patología tropical, etc. 1 (1):93-110.

2) R. Pollitzer (1954) Plague. WHO Monograph Series No. 22.

3) P. Cova García and Elizabeth Tallaferro (1959) Pulgas más comunes de Venezuela. Arch. venezolanos de patología tropical, etc. 3 (1): 327-348.

4) J. M. de la Barrera (1960) Resumen de los resultados de las investigaciones sobre peste efectuados en Venezuela desde el 19 de septiembre de 1959 hasta el 18 de febrero de 1960. (Unpublished typescript)

5) Idem (1960) Informe al Gobierno de Venezuela sobre peste. (Unpublished typescript)

6) WHO Weekly Epidemiological Records (1960-March, 1962), passim.

SECTION D

REVIEW AND EVALUATION OF THE PRESENT STATUS OF PLAGUE IN ECUADOR AS OBTAINED FROM THE TECHNICAL LITERATURE AND OFFICIAL REPORTS

History and recent incidence of plague

In marked contrast to what could be recorded in the case of Venezuela, the history of plague in Ecuador is rather involved. Dealing with this problem, Jervis Alarcón distinguished three phases, namely (1) Invasion of the seaports and other settlements in the coastal provinces; (2) spread of the infection by the railway system to the mountainous provinces of the interior which, taking place during the period from 1909 to 1939, successively led to the appearance of the disease in the provinces of Chimborazo, Tungurahua and Cañar, and finally in the small settlement of Guaytacama in the province of Cotopaxi (north of Tungurahua Province), the northernmost locality reached by plague in Ecuador; (3) penetration of the infection into the rural areas, taking place also in the southern province of Loja, in which plague, spreading by continuity from adjacent Peruvian areas, became entrenched during the period from 1918-1926.

The first of these three phases commenced in 1908 when the importation of plague by the sea-route led to an entrenchment of the infection among the rats of Guyaquil, followed soon by the appearance of manifestations of the disease in man. As a consequence of this fateful event, during the period from 1908 to 1913 almost all coastal towns of Guayas and Manabí provinces became plague-infected and the disease also appeared in the settlements of the coastal province of El Oro and the inland areas of Los Rios Province.

Generally speaking, plague in the coastal areas of Ecuador began to abate in 1924 and disappeared in 1930. However, owing to an importation from Chimborazo Province, the infection again flared up in Guayaquil in 1935 and persisted there until 1939 when the last human case in that port was recorded. Guayaquil has since then remained free from plague even though its rat population continues to be conspicuous. However, up to the present it came to a repeated reappearance of plague in other coastal localities, most frequently in El Oro Province where, according to Jervis Alarcón, 16 cases were recorded in 1939, 3 in 1940, 4 in 1950 and one in 1954 (community of La Libertad). These manifestations seemed to be due to an importation of infected fleas in bags of merchandise from Loja Province (Jervis Alarcón).

In 1954 the island of Puná, situated in the gulf of Guayaquil, became the scene of a most noteworthy outbreak which, according to the just mentioned observer, appeared to be due to an importation of the infection in goods from the seriously affected Loja Province rather than from El Oro. Notwithstanding this mode of the infection, the common rats (R. norvegicus) of Puná town did not become involved,

whereas a violent epizootic broke out among the wild rodents of the island, the plague nature of which was proved through positive findings in three species - Sigmodon puna, Oryzomys xanthaeolus and an unidentified species of Graomys (De la Barrera). The purely sylvatic nature of the outbreak is also proved by the fact that all 8 persons involved in it had contracted the disease when staying in the fields to collect wool from the silkcotton tree (the seeds of which form an attractive pabulum for the rodents). Drastic efforts to control the situation through a campaign against the wild rodents were crowned by success, these animals proving to be practically absent when De la Barrera visited the island in 1957. However, an exportation of the wool led to two plague cases recorded in 1955 at San Lorenzo, Cantón of Guayaquil, in Guyas Province.

The information on recent plague manifestations in the coastal areas of Ecuador may thus be summarized:

Year	Locality	Nature of Outbreak
1956	Jipijapa, Manabí Province	The occurrence of 7 plague cases in this important inland trading center stood apparently in causal connexion with the importation of materials for the manufacture of gunny-bags from Chimborazo Province.
1957	Vinces, Los Rios Province	Though no details are known, the infection of this important center of the trade in cacao, leading to 11 human attacks, undoubtedly also originated in Chimborazo Province.
1960	Pinas Cantón, El Oro Province	The occurrence of 5 plague cases in this locality has been recorded in the WHO wkly. epidem.Records, 36 (1961)8:84.
1961	El Oro Province	To judge from preliminary figures published in the Wkly. epidem. Record for 1961 and Jan/March 1962, during 1961 the occurrence of plague was notified in the Zaruma and Pinas Cantóns.
	Manabi Province	As mentioned in the 1961 Record, No. 40:429 and No. 50:525, an outbreak due to a rat epizootic and involving 71 persons took place in the port of Manta in August and September. In connexion with this epidemic two plague attacks were recorded in Quito. Towards the end of the year the appearance of plague in the Portoviejo and Rocafuerte Cantóns was reported.

Year	Locality	Nature of Outbreak
1962	Manabi Province	Plague cases continued to occur in the Rocafuerte Cantón and the Sucre Cantón began to be involved (Wkly. epid. Record 37(13):157).

For the convenience of record it appears to be indicated to deal with the plague manifestations in the provinces of Tungurahua and Cañar before devoting attention to the earlier affected Chimborazo Province.

As reported by Jervis Alarcón, plague appeared first in Tungurahua in 1926 in the city of Ambato, which once more recorded an epidemic, involving about 100 persons, in 1929. The disease then seems to have been absent from the province until 1956 when, probably imported through goods or infected domesticated guinea-pigs from an adjacent area of Chimborazo Province, it appeared in a rural area situated some kilometers southeast of Ambato. All the 12 attacks in the first affected area as well as two afterwards recorded in Ambato city seem to have stood in casual relation to the infection of domesticated guinea-pigs. There was only one death.

As can be gathered from the WHO weekly epidemiological Records, the occurrence of plague in the Tungurahua Province was again notified in 1960 and 1961. In the former year the Cantón of Pillaro seems to have been slightly affected, while in June 1961 some cases were recorded in a rural parish of the Ambato Cantón. It deserves attention that, according to a brief statement by Jervis Alarcón, plague was endemic in Tungurahua Province in an area of 40 km^2.

The province of Cañar, south of Chimborazo Province, became first plague-affected in 1933, when about 200 attacks with a high mortality were recorded in three localities. The disease reappeared there in 1945 (9 cases with 4 deaths). Afterwards 10 cases with 6 deaths were notified in 1951 at Las Postes, and 8 with 3 fatalities at Tambo. While the earlier outbreaks were evidently due to an importation of the infection from Chimborazo, that of 1953 was supposed to stand in relation to the importation of goods from the severely affected Cariamanga area in Loja Province. The size of the affected areas in Cañar totalled according to Jervis Alarcón 60 km^2.

Dealing with the plague situation in Chimborazo Province, Jervis Alarcón furnished the following valuable general information:

The province has a size of 5784 km^2 and had, according to the 1950 census, 218.130 inhabitants, 72.61% of whom lived in the rural areas. The altitude of the various regions varied from 1000 to 3000 meters above sea level, the temperature from 7° - 18°C. The weather was apt to be changeable even within one and the same day. The dry season lasted from June to December, the rainy period from December to May.

Plague, undoubtedly imported from the coastal areas, with which the Chimborazo Province is connected by rail, appeared there in 1909, first affecting the towns but becoming rural in character in 1940. The case incidence of the disease during the first phase (1909-1939) was, according to De la Barrera, 1420 as against an occurrence of 269 cases from 1940-1960. Some details on the manifestations of the disease during the period from 1946-1956 were set forth by Jervis Alarcón as follows:

Year	Districts affected	Foci	Cases	Year	Districts affected	Foci	Cases
1946	2	2	7	1952	2	7	18
1947	4	5	9	1953	1	1	4
1948	1	1	1	1954	7	14	27
1949	0	0	0	1955	3	4	10
1950	2	2	4	1956	3	6	10
1951	4	14	25	Totals		56	115

According to Jervis Alarcón the affected areas in the Chimborazo Province, now all situated in rural regions, have a total size of 400 km^2.

The recent plague incidence in the province was reported as follows in the WHO Weekly Epidemiological Record:

Year	Cantóns affected	Case incidence per cantón	Annual Total
1957	Guano	12	12
1958	Guano	7	
	Riobamba	1	8
1959	Guano	11	
	Riobamba	7	18
1960	Guano	15	
	Riobamba	8	
	Alausí	10	33

N.B. As far as could be ascertained thus far, plague reappeared in 1961 in the Guano and Riobamba Cantóns.

Characteristic for plague in the Chimborazo Province were

(1) A high mortality, amounting according to Jervis Alarcón to 85.7% in 1946, 64% in 1951 and 20% in 1956;

(2) A high incidence of pneumonic plague, due no doubt to the prevailing climatic conditions which induced the people to crowd together in their houses.

The province of Loja which, lying in the South of Ecuador, is adjacent to Peru, comprises according to Jervis Alarcón a territory of 9926 km^2 and had in 1950 a population of 216,802, almost 70% of the inhabitants living in the rural areas of the province. It consists of

(a) a low zone, 200-800m above sea-level with a temperature of 18°-30° in the shade, and (b) a mountainous part, 1500-2500m high with a temperature of 8°-19°C. It is important to note that the dry season, lasting from May to December, is the period of the year favourable for the occurrence of plague in the province, in which an area of about 3000 km^2, situated to a larger part in the low-lying zone, is affected by the disease.

As has been noted above, the infection infiltrated into Loja Province during the period from 1918-1926, the stages of this process having been thus described by Macchiavello (1957):

1918-21 - Plague became manifest in the Cazaderos - Alamor area;
1923 - The presence of the disease in the Cantón of Celica was suspected;
1925 - The occurrence of plague was noted in the cantóns of Catacocha (Paltas), Gonzanama and Cariamanga (Calvas);
1926 - Loja city was reached by the infection.

Considering this evolution, Macchiavello entertained no doubt that the appearance of plague in the Loja Province was due to an extension of the previously existing sylvatic focus in the Lancones district of Peru into Ecuador, resulting in the entrenchment of the infection among the wild rodents in a large area situated on both sides of the frontier between the two countries.

As stated by Macchiavello, during the period from 1925 to 1948 1617 plague cases with 864 deaths were noted in Loja Province, distributed as follows:

Cantón	Cases	Deaths	Cantón	Cases	Deaths
Alamor	305	135	Macará	67	33
Celica	230	125	Cariamanga	266	139
Catacocha	372	188	Gonzanamá	210	157
Loja	46	13	Amaluza	121	74
			Totals	1617	864

The incidence of the disease in Loja during the period from 1946 to 1956 was thus characterized by Jervis Alarcón:

Year	Districts affected	Foci of human plague	Foci of rodent plague	Cases	Deaths
1946	7	18	?	46	13
1947	4	7	?	17	1
1948	6	20	?	38	2
1949	7	17	?	20	3
1950	6	11	?	21	3
1951	5	9	?	10	1
1952	9	20	?	41	3
1953	6	28	11	64	15
c/f	50	130	?11	257	41

Year	Districts Affected	Foci of human plague	Foci of rodent plague	Cases	Deaths
b/f	50	130	?11	257	41
1954	9	34	1	111	8
1955	2	2	?	3	1
1956	7	30	5	42	3
Totals	68	196	?17	413	53

Dealing in the text of his article with the distribution of plague in Loja during the quinquennium from 1952 to 1956, Jervis Alarcón stated:

> "The spots (lugares) in these last 5 years are: in the eastern sector 4 out of 9 of the Loja District; 4 out of 8 in La Toma; 4 out of 6 in Gonzanamá; 19 out of 23 in Sozoranga; 18 out of 22 in Cariamanga; all 7 of Amaluza, all new; in the western sector 5 of the 7 lugares of Chaguarpamba; 13 out of 26 in Celice; 9 out of 19 in Alamor; 7 out of 11 in Pindal; 7 out of 13, all new, in Zapotillo".

According to this observer most severely affected were the districts of Cariamanga, Sozoranga, Catacocha, Celica, Alamor and Amaluza, the persistence of the infection among the wild rodents in them being "the true cause of the rural endemicity" in Loja. This is certainly a clue deserving great attention.

As added by Jervis Alarcón, the plague mortality in the province of Loja showed a marked decrease towards the end of the period considered by him, as shown by the following figures:

Period	Total cases	Recovered	Died	Mortality %
1926-1936	1479	928	551	37.2
1936-1946	906	611	295	32.6
1946-1956	410	369	41	11.1

The comparative benignity of plague in Loja was also evidenced by the rarity of the pneumonic type, only one small outbreak of lung pest having been recorded in the Catacocha District in 1939 (7 victims).

Data on the recent incidence of plague in Loja Province culled from the WHO Weekly Epidemiological Records, may thus be set forth:

Year	Plague Cases	Districts involved
1957	62	No details
1958	13	Calvas (2), Cariamanga (1), Celica (9), Macará (1)
1959	19	Catacocha (1), Celica (4), Loja (7), Macará (1),Paltas (6)
1960	36	Calvas (17), Celica (1), Macará (11), Paltas (7)

N.B. Preliminary data for 1961 indicate a recurrence of plague in the cantóns of Calvas, Celica and Paltas.

Observations on rodents and other ecologically important mammals

'Domestic' species

As far as can be judged from the rather scanty information available in regard to the occurrence of the common rats in the plague-affected areas of Ecuador, these rodents, while abundant in the settlements of the coastal areas (where R. norvegicus appears to be the most frequent species), are irregularly distributed in the interior, especially in the mountainous districts. Macchiavello maintained in this connection, in his study on the province of Loja, that the reason for this unequal distribution of the rats and their absence from still many settlements seemed to be that these rodents began to penetrate only since 1920 in the remote interior areas of Ecuador, especially those lying away from roads and railways. He illustrated the irregular distribution of the three rat species in the towns of Loja Province by the following table, based upon the results of captures made from 1938 to 1942:

Towns	R. rattus	R. norvegicus	R. alexandrinus
Loja	14	22	2
Guachanamá	0	0	2
Federico Páez	2	0	10
Zapotillo	0	0	0
Cazaderos	1	1	2
Sabiango	0	3	0
Sozoranga	0	5	4
Chaguarpamba	35	47	89
Cariamanga (Calvas)	24	53	109
Gonzanamá	17	56	260
Celica	66	72	173
Amaluza	22	329	696
Catacocha (Paltas)	6	335	439
Macará	5	281	293
Alamor	4	50	178
Totals:	196	1254	2257

To judge from these data, R. alexandrinus was most frequent among the urban rats in Loja, while R. rattus was rare. As stated recently by Jervis Alarcón, R. alexandrinus was also the prevalent rat in Chimborazo Province.

The irregular distribution of the common rats in Ecuador was confirmed by De la Barrera (1957) who, however, made almost all of his observations in Loja Province and admitted moreover that he had devoted his main attention to the wild rodent species. As can be gathered from De la Barrera's report, he had met in Loja with all three subspecies of

R. rattus (rattus, alexandrinus and frugivorus) as well as with R. norvegicus. While the latter was of rather domestic habits, never leaving the immediate vicinity of the houses, R. rattus was trapped not only in but also round the settlements within a radius of 1200 meters.

Dealing with the role played by the rats in the plague manifestations of the presently active Ecuadorian foci, De la Barrera (1957) (a) referred to a small 1956 outbreak taking place at Chinchil, in a region where the Rattus species were absent (domesticated guinea pigs serving as the means to convey the infection to man); and (b) stated in a general manner that he had not met with rats in the plague-infected houses. Maintaining in his final report (1961) that the common rats of Ecuador had become resistant to plague, De la Barrera came to the conclusion that "there was evidence that in the actual moment the importance of Rattus as reservoir and spreader of plague in Ecuador is secondary or nil." He was careful to add, however, that "the great murine communities parasitized by X. cheopis, surrounded by sylvatic (plague) infection constitute a tangible risk."

Mus musculus

The frequency of the house-mice in Loja Province is well illustrated by observations quoted by Macchiavello, according to which during the period from 1938 to 1942 not less than 49393 out of a total of 68566 captured rodents were M. musculus. In accordance with these data, De la Barrera stated that this species "was met with as usual, in the houses and in the open fields". However, Jervis Alarcón maintained that, though M. musculus was widely spread in Ecuador, existing also in the mountainous areas, still there were many communities which had not been invaded by these rodents or by Rattus, particularly in zones distant from roads and railways.

That the house-mice in Ecuador occasionally may be involved in the transmission of plague from the wild rodents to the houses has been confirmed by an observation made in 1956 by De la Barrera: In a house at San Fernando, a locality round which wild rodent plague was rampant, 12 plague-infected M. musculus were found. Since, however, before this discovery five people had contracted the disease, no doubt through the bites of wild-rodent fleas (Polygenis litargus) abounding in the house, it is possible that the infected mice were merely victims to the infection instead of playing a causative role in the human outbreak.

Domesticated rodents

That the domesticated guinea pigs, amply bred in the houses of the Ecuadorian plague foci and living in closest contact with the inhabitants, are apt to play an ominous role in the transmission of the infection from the wild rodents to man, has been proved on many occasions. An observation in point, made by De la Barrera, has been quoted above. Owing to the habit of the Indians to send such animals as presents to their friends, the guinea pigs may also become responsible for a spread of the infection

at distance. Another most dangerous practice is the absconding of guinea pigs from plague-affected houses or settlements, resorted to by the Indians whenever they fear that as a control measure these animals might be taken away from them and be destroyed.

Besides guinea pigs, also rabbits (Oryctolagus cuniculus) are kept in the houses of the Ecuadorian plague foci, but this is done on a much more limited scale. Moreover, contact of man with them is less intimate than that with the guinea pigs (De la Barrera, 1957).

Wild rodents and lagomorpha

Fairly ample information on the wild rodents of Loja Province can be culled from the reports of De la Barrera and Jervis Alarcón, while the latter also furnished some supplementary data in point for the province of Chimborazo.

Most frequent among the wild rodents in both provinces are two species of cricetinae, Akodon mollis and Oryzomys xanthaeolus (95% of the captures of De la Barrera). Both are able to adapt themselves to a wide variety of habitations without predilection for any of them. They are both apt to invade the rural houses at night time. They differ in so far as O. xanthaeolus is more heavily infested with the fleas common to all the small rodents of Loja Province. Both species are highly susceptible to infection with P. pestis and thus apt to be involved in widely spread acute plague epizootics. As observed by De la Barrera in 1956 at Santa Ana, plague-affected rodents of both species may be found even inside rural houses.

No ecological data could be found regarding some other Oryzomys species, including O. longicaudatus and O. flavescens, as well as in regard to Phyllotis fruticicolus which, like the two above mentioned species of cricetinae, have been found naturally plague-infected in Ecuador.

Sigmodon peruanus, also belonging to the subfamily of cricetinae, though less frequent than A. mollis and O. xanthaeolus, is also widely spread in Loja Province. According to De la Barrera, it is moving about rather slowly and, therefore, is but rarely met with in the houses. Like its congener, Sigmodon puna, it has been found naturally plague-infected.

The squirrel Sciurus stramineus nebouxi, a tree-inhabiting species, though generally of sylvatic habits, not rarely enters the houses in daytime in search of morsels of food. This species deserves great attention insofar as, being considered as comparatively resistant to infection with P. pestis, it has been suspected by Macchiavello to be the permanent plague reservoir in Loja Province, thus being responsible for the periodical appearance of acute epizootics in the highly susceptible cricetinae A. mollis and O. xanthaeolus. It is important to note in this connection that S. stramineus, which has been found to be infested at a low rate with the flea Polygenis litargus, is met with in more restricted locations than

the small Ecuadorian rodent species. Hence, should the role of S. stramineus as the principal plague reservoir in Loja Province be proved, the localities inhabited by it might form strongholds of the infection.

As suggested by Jervis Alarcón, in Chimborazo Province, Sylvilagus brasiliensis and allied species of lagomorpha, which are also comparatively resistant to plague, might play a role comparable to that of S. stramineus in Loja. It is noteworthy that Sylvilagus, a large animal hunted for the sake of its meat, also belongs to the species restricted in their habitat. Its specific flea is Hoplopsyllus manconis.

It deserves attention that, in strict contrast to the above discussed postulations, De la Barrera insisted that the Ecuadorian wild rodents "constituted by species which live together or enter in contact, are equally sensitive (to plague), are parasitized by the same fleas and constitute a homogeneous whole in regard to plague. The importance of each species in the transmission depends solely upon its abundance and its habits."

De la Barrera maintained also that the large rodent species (Cuniculus, Dasyprocta, Sylvilagus and Sciurus) apparently did not play an important role in the ecology of plague. It will be the object of further investigations to decide whether his contentions or, perhaps in a modified form, the more plausible suggestions of Macchiavello and Jervis Alarcón are valid.

Observations on fleas

Rat fleas

Dealing in a general manner with the occurrence of X. cheopis in Ecuador, Jervis Alarcón stated that in the coastal areas up to an altitude of 1200 m., this flea was practically alone met with on the common rats and was also solely responsible for the conveyance of the infection in the past epizootics and epidemics. In the mountainous areas of the interior, X. cheopis was considerably less abundant than on the coast, not occurring in numbers sufficient to cause widespread spizootics followed by epidemics. It was not found in localities above about 3000 m., being replaced in the highly situated areas of Chimborazo Province by Nosopsyllus londinensis. As stated by Jervis Alarcón, this flea, frequent in altitudes of above 1400 m., though a less efficient vector than X. cheopis, seemed at low temperatures more suited than the latter to serve as a reservoir of P. pestis and consequently was in Chimborazo Province the flea responsible (causante) for "the maintenance or endemicity of plague" -- playing thus a role analogous to that of Polygenis litargus in Loja Province.

It is striking to note that De la Barrera, reporting on his investigations in Loja Province, listed among almost 12000 fleas examined by him only four X. cheopis, apparently 3 in the nests of not determined rodents and one on a lot of 16 domesticated guinea pigs[a]. It has to be

---

[a] Later in his report he also mentioned 2 cheopis fleas found on R. norvegicus.

noted, however, that (a) as already mentioned, this observer made his studies on wild rodents rather than on rats and (b) to judge from Macchiavello's article, during the plague outbreaks observed by him in 1943 in the Cariamanga Cantón of Loja Province, X. cheopis was the prevalent flea on the rats (mainly R. alexandrinus) and thus evidently served as the vector of the infection. Further studies, showing the comparative frequency of X. cheopis in the various cantóns of Loja Province, seem thus most necessary.

Mouse fleas

As stated by Jervis Alarcón, the fleas met with on the house-mice of Ecuador belonged to the species Leptopsylla segnis, Tiamastus cavicola, Pulex irritans and (rarely) X. cheopis. Among 11 fleas collected by De la Barrera in Loja Province from M. musculus, 6 were L. segnis, 4 T. cavicola and 1 N. londinensis.

Fleas of the domesticated guinea pigs

Referring to the fleas of the domesticated guinea pigs, Jervis Alarcón recorded the following figures:

Loja Province		Chimborazo Province (Riobamba)	
P. irritans	67.7%	T. cavicola	69.5%
P. litargus	32.1%	Hectopsylla eskeyi[b]	29.4%

The flea incidence on Cavia cobaya observed in Loja Province by De la Barrera was as follows:

P. irritans	1804	Polygenis sp.	2
T. cavicola	595	X. cheopis	1
H. eskeyi	45	L. segnis	1
P. litargus	44	Cten. felis	1
		Total:	2493

The occurrence of Polygenis litargus, the wild-rodent flea mainly responsible for the transmission of plague in Loja Province on the domesticated guinea pigs is of great significance. Tiamastus cavicola, the specific flea of the guinea pig, has been found naturally plague-infected in both Ecuador and Peru but is not considered as an efficient vector of P. pestis.

Fleas of the marsupialia

That marsupialia (Monodelphis and Didelphis), because having ample contact with the wild rodents but also frequenting the houses, are apt to

---

b) This species and also H. suarezi have been found naturally plague-infected in Ecuador. Both these fleas were also found on common rats.

play a role in bringing infected fleas to the immediate vicinity of man, is confirmed by the following flea list furnished by De la Barrera for Didelphis marsupialis:

Polygenis litargus+	4	Rhopalopsyllus cacicus	32
P. bohlsi bohlsi+	2	Ctenocephalides felis felis+	3
Adoratopsylla intermedia copha+	3		
			44

Neotyphloceras rosenbergi, found by De la Barrera on Didelphis azarae, has also been found naturally plague-infected in Ecuador.

Wild rodent fleas

Amply dealing with the wild rodent fleas in the province of Loja, De la Barrera furnished the following general tabulation:

+ Found naturally plague-infected.

Flea species	Found on: O. xanthaeolus No	%	Akodon mollis No	%	Other wild rodents No	%	Rodent nests No	%
P. litargus+	439	76.4	757	45.7	343	71.7	4878	71.4
P. bohlsi bohlsi+	43	7.4	248	20.4	78	16.3	1557	22.6
P. brachinus+	63	10.9	23	1.8	1	0.2	350	5.0
Polygenis n. sp	17	2.9	58	4.7	8	1.6	53	0.7
N. rosenbergi+	6	1.0	34	2.8	37	7.7	16	0.2
Plocopsylla n. sp+	6	1.0	1	0.1	-	-	-	-
P. phobos	-	-	37	3.0	-	-	-	-
Pleochaetis dolens quitanus+	-	-	42	3.4	-	-	-	-
Tunga n. sp.	-	-	1	0.1	-	-	-	-
R. Cacicus	-	-	1	0.1	-	-	-	-
Pulex irritans n. s. sp.	-	-	1	0.1	1	0.2	-	-
P. irritans+	-	-	2	0.2	-	-	4	0.05
C. felis felis+	-	-	5	0.4	1	0.2	1	0.01
Craneopsylla minerva	-	-	1	0.1	-	-	-	-
A. intermedia copha	-	-	1	0.1	7	1.4	1	0.01
Cleopsylla monticola	-	-	-	-	2	0.4	-	-
Ctenidiosomus spillmanni	-	-	-	-	-	-	2	0.02
Dasypsyllus lasius	-	-	-	-	-	-	23	3.3
X. cheopis	-	-	-	-	-	-	3	0.04
Totals	574		1212		478		6888	

+) Found naturally plague-infected.

Commenting upon his findings, De la Barrera stated that Polygenis litargus was not only the flea most frequently met with on the wild rodents of Loja Province but was also found on the common rats of the rural areas and on the domesticated guinea-pigs. Though considering this flea as a probable vector, conveying P. pestis not only from rodent to rodent but also to man, De la Barrera maintained that its role had not been definitely proved. It is, however, important to add that these doubts were soon set at rest, Macchiavello recording one year after the publication of De la Barrera's report experimental findings confirming the capability of Polygenis litargus to convey the infection. Though with some restrictions, he considered it as a "splendid" plague vector. One must, however, agree with De la Barrera that most probably this flea is not the only vector of the infection in Loja Province, and that thus there is an urgent need to test the capability of the other wild-rodent fleas met with there, paying prime attention to the several species which, like P. litargus, have been found to harbor P. pestis under natural conditions.

It is disappointing to find that, in contrast to the quite ample prima facie evidence available in regard to the Loja fleas, information on the species probably concerned in the conveyance of plague in the province of Chimborazo is not only quite scanty, but also contradictory. Jervis Alarcón is evidently of the opinion that in that plague area N. londinensis, found on Oryzomys xanthaeolus and Akodon mollis besides on the rats, plays a role analogous to that of P. litargus in Loja. However, in his 1961 report De la Barrera made the following statement:

> "Though our information on the Siphonaptera of the northern zone (Chimborazo) seems incomplete, there can be no doubt that the flea species are not the same as in the southern zone. The principal difference is that Polygenis litargus, the predominant and probably the vector species in the province of Loja, is not met with above 2200 meters of altitude, being replaced in Chimborazo by Pleochaetis dolens quitanus."

It is clear, therefore, that further studies on the occurrence and comparative importance of these two fleas are urgently called for. Great attention must also be paid to Hoplopsyllus manconis, the specific flea of Sylvilagus brasiliensis which, as has been noted above, is in the opinion of Jervis Alarcón possibly the fons et origo mali in Chimborazo.

It has to be added that the present reporters have not been able to find any information on the rodents and fleas implicated in the plague manifestations in the provinces of Tungurahua and Cañar.

Ecology and epidemiology of plague

Though, as has been noted already on several occasions and will be further discussed below, still many gaps in its knowledge exist, it may be claimed nevertheless that the general features of the ecology of plague in Ecuador have become clear. There can be no doubt that in the presently affected areas of that country plague is basically of a sylvatic nature, the persistence of the infection in wild rodents being

responsible for the occasional appearance of the disease in the 'domestic' rodent fauna and in man. Though, to judge from the information available only for the two worst affected areas, the provinces of Loja and Chimborazo, several species of wild rodents and lagomorpha have been found involved in the plague manifestations, at first glance two species of cricetinae, A. mollis and O. xanthaeolus appear to be most seriously affected. There can be no doubt that in these two most numerous and widely spread species infection by P. pestis plays the role of a population regulator, the disease becoming periodically rampant among them when, for reasons it would lead too far to discuss here, the population density of the herds has reached high levels. The resulting tides of widespread acute epizootics are followed by periods during which owing to the decimation of the herds the infection finds little if any fuel for its spread or even persistance. It is under these circumstances a plausible idea that the above mentioned cricetinae species do but periodically suffer from plague, the infection being permanently harbored by other rodents. As has been stated, this has been actually postulated, Sciurus stramineus being incriminated as the permanent plague reservoir in Loja, while an analogous role has been ascribed in Chimborazo Province to Sylvilagus brasiliensis. As will be discussed in the following part of this report, observations made in Peru lend some support to the view that Sc. stramineus might be capable of serving as a permanent plague reservoir. However, as far as the present reporters are aware, no proof whatsoever has been obtained that an identical role might be played by S. brasiliensis. Nevertheless, one may accept it as a working hypothesis that not the cricetinae, but other species of rodents or lagomorpha constitute the permanent plague reservoirs in the Ecuadorian plague foci. To confirm or to establish which species are actually involved, is one of the urgent tasks of further investigations, because only after their discovery will it be possible to determine whether and where within the affected areas strongholds of plague exist. The recognition of such 'elementary foci', the existence of which seems to have been hinted at by Macchiavello and Jervis Alarcón, would be of great importance for an adequate prevention and control of plague.

In contrast to the above discussed problem, for the attempted solution of which in part hypotheses rather than established facts had to be adduced, the manner in which plague finds its way from the wild rodents to man has in the main been elucidated.

As can be gathered from De la Barrera's reports, human infection has been sometimes contracted by persons working or staying in the foci of wild-rodent plague. No doubt, however, an intradomestic infection of man is the rule in the presently affected areas of Ecuador. This may be effected in various ways. Thus, as confirmed by an observation of De la Barrera, plague-infected rodents may occasionally penetrate into the houses, their fleas thus becoming capable of conveying the disease to man. Infected wild-rodent fleas may also be brought into the houses in other ways, particularly by the marsupialia frequenting the human habitations as well as their vicinity, and may then directly attack man. It may be noted in this connexion that De la Barrera was able to cultivate P. pestis from 2 out of 10 Polygenis litargus fleas collected in a plague-infected house.

Ample experiences have shown, however, that often the manifestation of plague in man is preceded by the infection of the common rats, the domesticated guinea-pigs or occasionally the mice living most or all the time in the human habitations. While there can be no doubt that wild-rodent fleas are instrumental in conveying the infection to these animals, pending further investigations it is difficult to decide which fleas function as the vectors of P. pestis from the latter to man. It should be noted in this connexion that (a) X. cheopis, a notoriously efficient vector, appears to be scanty in the presently plague-affected areas of Ecuador and altogether absent in their higher parts; (b) N. londinensis, found on the rats in Chimborazo Province, is not a highly efficient vector; and (c) it seems to be unknown, to what extent the common rats are infested with wild-rodent fleas. It can be pointed out, however, that according to the above quoted observations of Jervis Alarcón, the domestic guinea-pigs of Loja Province were to a remarkable degree infested with Polygenis litargus and it seems likely, therefore, that this flea was instrumental in conveying the infection from guinea-pig to guinea-pig and from them to man. That P. irritans, according to Jervis Alarcón, the predominant flea of the guinea-pigs in Loja, played an important role in this respect, seems at the present state of knowledge on this species less likely.* Whether Hectopsylla eskeyi, found besides the rather inefficient vector Tiamastus cavicola on the guinea-pigs of Chimborazo Province, is of importance in the conveyance of plague, remains to be seen.

Though instances of the appearance of two or more cases in one and the same house to be frequent (occurring, according to De la Barrera in 1956 in 42% of the affected habitations), a spread of the disease from man to man through the agency of infected fleas seems not at all likely.

That fundamental differences in the ecology of plague exist between the Loja and Chimborazo provinces, is confirmed by the fact that the seasons of the appearance of human plague in the two areas are not identical. Jervis Alarcón stated in this respect that

> "in Chimborazo, between 1946 and 1956, the majority of the cases appeared in the hottest months with a high relative humidity, from February to May, and the rare cases appeared in the summer months of no excessive heat between August and December, whereas in Loja (plague) became intensified after the rainy season, during the harvest period, i.e. from May to December."

Recommendations for further investigations

As already repeatedly stated in the foregoing pages, in many respects the information available in regard to the ecology and epidemiology of plague in Ecuador is still incomplete. There exists thus an urgent need for further investigations, most essential among which seem the following:

---

* This point will be further discussed in the report on Peru.

Coastal provinces

Though since the final dissapearance of plague from Guayaquil in 1939 the disease does not seem to have any more a permanent foothold in the coastal provinces of Ecuador, it had to be noted that within recent years repeatedly long-distance sprints of the infection from the endemic foci in the interior have led to the appearance of the disease at or near the sea-coast. To judge from the perhaps not complete available information, these outbursts could become quite serious, as in Manta for instance, where a considerable rat-epizootic preceded and accompanied the manifestation of the disease in man. The fact that such an epizootic could evolve, shows clearly that the resistance of the Ecuadorian rats to plague is not as universal or not as permanent than it has been postulated. An appraisal or re-appraisal of the plague-receptivity of the rat-population in the various communities of the coastal provinces (in the first line in the ports and other traffic centers) and as far as possible also in representative rural areas would, therefore, be most desirable. As shown by large-scale investigations in point made in India, it would be possible to make the susceptibility tests in a central laboratory, to which batches of rats from the various locations are forwarded. An exact knowledge of the plague history in each of these would be of great importance for the evaluation of the results.

Advantage ought to be taken of the trapping of these rats to assess

(a) the comparative density of the rat populations in communities of different size and in the rural areas;

(b) the relative frequency of the two rat species and (though this is of lesser importance) of the subspecies of R. rattus;

(c) The occurrence and frequency of the various species of rat-fleas, specially of X. cheopis, in the various locations studied.

(Since, however, the frequency the rat-fleas may be subject to marked seasonal changes, it would be highly desirable to continue such studies, at least in selected communities, for a period not less than a whole year).

Detailed instructions for the trapping of rats, the collection of fleas and the transport of both to the central laboratory, suitable under the local conditions, will have to be framed and issued.

Endemic areas of the interior

1) Ecological and epidemiological investigation

To judge from the available information, the endemic foci in the provinces of Loja, Chimborazo, Tungurahua and Cañar have been found

well delimited. Since, however, the observations in point were made some years ago, it will be important to determine in close cooperation with the workers of the National Plague Prevention Service whether the boundaries of the endemic areas are still the same at present or whether the foci have shown a tendency to increase or decrease in size. If possible, large-scale maps, showing the present boundaries of the endemic foci should be prepared.

As has been mentioned above, it appears that within the endemic area of Loja Province there exist districts with a particularly serious plague situation, in which evidently the infection shows a marked or even permanent tendency to become recrudescent. Every possible effort should be made to confirm the continued existence of such centers of the infection, correlating observations on the incidence of human plague (upon which the information presently available in this respect seems to be mainly based) with particularly intensive studies on the rodents and fleas. Efforts should also be made to determine whether such centers of the infection exist also in the other three affected provinces, specially in that of Chimborazo.

All human manifestations met with by the study group ought to be made the subject of thorough investigations. In the first line efforts should be made to establish which rodents and which fleas were responsible for the infection of the patients. The presence of plague in them ought to be confirmed invariably through adequate laboratory tests and the strains of P. pestis isolated from the sufferers ought to be kept in a lyophilized state for further study.* Moreover, efforts ought to be made to detect the occurrence of subclinical forms of the disease through clinical and serological surveys of groups of the population actually or potentially under the risk of infection.

2) Investigations on rats, house-mice and their fleas

As has been discussed, the information on the occurrence of the two species of rats and also that on the frequency of the house-mice in the endemic areas is rather incomplete. It is, therefore, most important to study the present distribution of these rodent species in the endemic areas or, if possible, in the four affected provinces as a whole. The zones inhabited by these animals might with advantage be indicated in the maps of the endemic areas.

All rats and house-mice found dead or trapped within the endemic areas ought to be dissected and examined for the presence of plague or of past infection with P. pestis. Advantage ought to be taken for this purpose not only of macroscopic and microscopic observations, cultivation and pooling tests but also of serological tests. The latter as well as the pooling tests and perhaps even the cultivations might be made in base laboratories, to which the adequately organs and the blood or serum of the dissected animals are forwarded.

---

* This naturally also holds true of the strains isolated from rodents and fleas.

It would be further essential to ascertain whether or to what extent the rats within the endemic areas are resistant to infection with P. pestis. If, as will be probably inevitable, the animals have to be sent for this purpose to a base laboratory, they would have to be carefully de-flead before they are dispatched.

The rather scanty information on the flea fauna of the rats in the endemic areas must be supplemented by further large-scale surveys. Particularly important is (a) to establish to what extent the rats of Loja Province are infested with X. cheopis and which fleas parasitize the rats in its absence; (b) to confirm that in Chimborazo Province N. londinensis is the specific rat-flea; and (c) to determine which species infest the rats in the endemic areas of the Tungurahua and Cañar provinces.

While the vector efficiency of X. cheopis may be taken for granted, it would be important to study the vector capacity of the fleas replacing it, particularly of N. londinensis. Analogous studies ought to be made also with P. irritans, frequent on the domesticated guinea-pigs of Loja Province and with Tiamastus cavicola and specially with Hectopsylla suarezi, infesting these animals in Chimborazo Province.

3) Investigations on wild rodents and lagomorpha

As will be gathered from the statements made earlier in this report in regard to the observations on wild-rodent plague in the Ecuadorian endemic areas, the most important problem yet to be solved is which of the several species found to suffer from natural plague form the permanent reservoir of the infection. Thus far it has been assumed that in Loja Province the squirrel Sciurus stramineus nebouxi is the fountainhead of the infection, while in Chimborazo an analogous role of Sylvilagus brasiliensis has been suspected*. Apparently the main argument in favor of these species was that they were supposed to be less susceptible to plague than the cricetinae. It would seem, however, that this claim is not supported by convincing evidence. Moreover, the present reporters have to point out that the supposed resistence of a rodent species to plague should not be considered as the sole criterion of its capability permanently to harbor the infection. Experiences in other plague-affected countries have shown that a more or less refractory state to infection with P. pestis may be of a racial or seasonal nature rather instead of being inherent in the species in question as a whole all the time. Hence, while it is certainly important to make exact comparative studies of the degree to which the various species of the Ecuadorian wild rodents are amenable to P. pestis infection, a decision whether or not the above mentioned or other species are the permanent reservoir of the disease should also be based upon careful considerations of their ecology and the constancy of the occurrence of natural plague in them. As suggested with much reason by Macchiavello, the best times for observations of the latter kind are the periods or seasons during which wild rodent plague

* No statements in point have been made in the case of the Tungurahua and Cañar provinces which form in this as well as in many other respects still a terra incognita.

in general is at an ebb. Findings of the continued existence of plague in a rodent species at such times would go a long way to incrimate it as a permanent reservoir of the infection. While paying due attention to these investigations, a careful watch must be kept also on the trend of the disease in the cricetinae in which epizootics become periodically rampant. Since, as has been discussed above, there is every reason to assume that these high tides of the infection and the subsequent ebbs are correlated with fluctuations of the population density of the animals, constant or at least periodical surveys ought to be made to assess their frequency. Well organized observations of this kind would form an easy means to forecast the appearance of epizootics, during which the risk of human infections is highest.

In order to ascertain the presence of plague in the wild rodents it is necessary (a) carefully to examine all animals found dead with the aid of macroscopic and microscopic observations, cultivation and, if indicated, animal experiments; (b) to watch for the occurrence of incipient or latent infections with P. pestis by trapping* adequate numbers of the rodents and, perhaps after a period of observation in the live state, sacrificing them and subjecting them to the above mentioned laboratory tests (substituting pooling tests with their organs to individual animal experiments). Another promising method would be to make tests with the sera of apparently healthy animals. As far as possible, the above outlined tests should be made in a base laboratory, to which the adequately preserved organs and the blood serum of the animals are forwarded. It is obvious that, whenever necessary, their skulls and skins should be preserved for identification tests.

A part of the trapped animals ought to be used for testing their susceptibility or resistance to infection with P. pestis. Since tests of this nature presuppose the availability of adequate laboratory facilities, consideration must be given to the possibility of forwarding batches of the animals to a base laboratory after they have been de-flead and kept for some time in quarantine. One must fear, however, that the high mortality frequent in wild rodents kept in captivity will militate against the implementation of these procedures. As soon as the importance of a species of rodents or lagomorpha for the permanent harborage of P. pestis has been established, a close study of its ecology, particularly of the limits of its habitat, must be made and the trend of the infection in the animals in question must be taken under constant observation. If the ecological conditions warrant this, a pilot study might be made to assess the possibility of eradication campaigns.

4) Investigations on wild-rodent fleas

To obtain an ample material of the Ecuadorian wild-rodent fleas for study is of utmost importance in order (a) to arrive at a better understanding of the comperative frequency of the various species on the different wild rodents or lagomorpha and in their nests as well as on

---

* In the case of species which are difficult to trap, killing the animals by an adequate method may become necessary.

liaison animals like the marsupialia and on the rodent species living in or near the houses; (b) to study at the same time the seasonal incidence of the various fleas; and (c) to assess the comparative importance of the different wild-rodent fleas in the transmission and the inter-seasonal carry-over of plague. Constant efforts should be made, therefore, not only to collect fleas from all rodents coming under observation but also to make systematic searches for these parasites in the rodent nests and harborages as well as in the houses where the guinea-pigs, veritable flea-traps, are apt to furnish an adequate material.

It is clear that the above outlined investigations would yield valid results only when the various flea species met with can be properly identified -- a task which unfortunately could by no means always be properly performed by the field workers. They should take care, therefore, to submit representative specimens of their collections to an expert for identification. It would be certainly best if for this advantage could be taken of the unique knowledge on the South-America flea species possessed by the custodian of the Rothchild flea collection in Tring, (England). Since, however, the results of his identifications would become available only with delay, it would be essential to attach a flea expert to the study group operating in South America.

To arrange for adequate tests on the vector capacity of the fleas is also difficult. No doubt this task could be performed most satisfactorily in one of the United States laboratories possessing special experience in this field. Nevertheless it would seem well to make preliminary tests of this nature in a base laboratory in Ecuador and to attempt at the same time to raise colonies of the various fleas, lots of which could then be sent to the United States for final vector studies.

When investigating the role of the wild-rodent fleas in the individual endemic areas of Ecuador, prime attention ought to be paid to the following problems:

(a) Loja Province- Further studies on the distribution and seasonal incidence of Polygenis litargus ought to be made and its vector efficiency ought to be confirmed through further tests. At the same time it should be determined through ecological investigations, laboratory tests and vector studies to what extent other species of wild-rodent fleas participate in the transmission of plague. Prime attention should be paid in this respect to the species already found naturally infected.

(b) Chimborazo Province - The distribution and seasonal incidence of Nosopsyllus londinensis, Pleochaetis dolens quitanus and Hoplopsyllus manconis should be thoroughly investigated and large-scale tests ought to be made to determine to what extent they are naturally plague-infected. It would also be essential to compare the vector capacity of these three species.

(c) Tungurahua and Cañar Provinces - Since no information on the flea fauna in the endemic areas of these two provinces could be elicited, it would be important to establish by what species the rodents met with are infested, to study the seasonal incidence of the fleas and determine through ample laboratory tests which of them are naturally plague-infected. The vector capacity of the species incriminated in this or other ways should then be studied.

It is hoped that for the drafting of a definitive plan for plague studies in Ecuador it will be possible to take advantage of the experiences gathered in the course of the investigations in point now envisaged for Peru.

References:

1) R. Pollitzer (1954) Plague. WHO Monograph Series No. 22 (Summary of the older literature).

2) A. Macchiavello, Estudios sobre la peste selvática en America del Sur: II (1957) Peste selvática en las zonas fronterizas de Peru y Ecuador. 1. Peste en la Provincia de Loja, Ecuador. Bol. Of. Sanit. Panameric. 43 (1): 1941.

IV (1958) Transmisión experimental de la peste por Polygenis litargus. Bol. Of. Sanit. Panameric. 45 (2): 122-131.

3) J.M. de la Barrera (1957) Informe presentado por la Oficina Sanitaria Panamericana, Oficina Regional de la Organización Mundial de la Salud, al Gobierno del Ecuador (Unpublished typescript).

4) Idem (1961) Informe al Gobierno de Ecuador sobre peste (Unpublished typescript).

5) O. Jervis Alarcón (1958) La peste bubónica: Problema de urgente resolución.

Rev. Ecuat. de Hig. y Med. Trop. 15 (1958) 3: 105-137

6) WHO Wkly. Epid. Records (1961-30 March 1962) passim.

SECTION E

REVIEW AND EVALUATION OF THE PRESENT STATUS OF PLAGUE IN PERU AS OBTAINED FROM THE TECHNICAL LITERATURE AND OFFICIAL REPORTS

History and recent incidence of plague

As summarized by Pollitzer, following its importation by the sea-route into the port of Callao in 1903, the disease spread along the coast of Peru, most of the principal ports becoming infected within two years, and eventually invaded 10 of the 20 departments of the country, as well as the three special provinces of Tumbes, Callao and Moquega. The situation became worst in the coastal department of Lambayeque, Libertad and Lima, as well as inland in Cajamarca. The total incidence of the disease may be summarized thus:

Period	Cases	Annual average
1903-1912	8865	886
1913-1922	6922	692
1923-1932	4642	464
1933-1942	1087	109
1943-1952	738	74
1954-1960	353	50
1903-1960	22607	2275

More detailed figures for the period from 1956 to 1960 may be set forth thus:

	Areas affected		Incidence		
Year	Department	Province	Per Province	Per Department	Annual Total
1956	Piura	Ayabaca	8		
		Huancabamba	16	24	24
1957	Piura	Ayabaca	6		
		Huancabamba	11	17	
	Ancash	Huaraz	11		
		Recuay	3	14	31
1958	Piura	C. Villar	3		
		Sullana	6		
		Ayabaca	24	33	
	Lambayeque	Lambayeque	7	7	
	Cajamarca	Hualgayac	4	4	
	Ancash	Huaraz	5	5	49*
1959	Piura	Ayabaca	14	14	
	Cajamarca	Hualgayac	18	18	32
1960	Piura	Ayabaca	15		
		Huancabamba	112	127	
	Cajamarca	Hualgayac	7	7	134

(See asterisk on page 2)

As can be gathered from the Wkly epidemiological Records published thus far in 1962, 63 plague cases were recorded from 22 October until 9 December, 1961 in the Huancabamba District and 47 cases from 31 December, 1961 to 10 February, 1962 in the Tabacones District, Jaén Province, Cajamarca Department.

How far this at first glance rather disquieting increase of the case incidence since 1960 is merely the result of an improved reporting system, it is impossible to decide.

Dealing in his classical study with the plague situation in Peru up to 1932, Eskey stated that the central part of the country, situated between the 7th and 13th degree of latitude and specially the areas between the 7° and 9° with an average annual mean temperature between 69°F (20.5°C) and 71°F (21.7°C) suffered most from the disease. There the infection spread rapidly to rural as well as urban communities, produced more cases than elsewhere in the country, and showed little tendency to disappear spontaneously. However, the degree of rat infestation of the houses was also of importance. Thus the ports of Paita in the north of Peru and of Mollendo in the south, though situated well away from the zone where the climate favored the spread of plague, suffered heavily because their wooden buildings were attractive to the rats. Lima on the contrary, though open to inroads of the infection as far as the climatic conditions were concerned, had a lesser morbidity than these two ports because of having better class houses.

Generally speaking, the annual plague epidemics in Peru tended to reach their peak during the summer months. However, there as elsewhere the plague seasons fell into an earlier period in areas where the winter months were warm than in localities with a colder climate.

Human plague in Peru was mostly bubonic, but one pneumonic outbreak, claiming 21 victims in the department of Junín is on record.

As described by Eskey, R. norvegicus, R. rattus and R. r. alexandrinus were common in the towns of northern Peru, while in the central and southern coastal areas as a rule Norway rats greatly exceeded the other two species. X. cheopis was the most common rat flea and, according to Eskey, the only important plague vector.

Recent manifestations of rat-caused plague

Information on further rat-caused plague outbreaks in Peru is rather scanty. Ramos Días referred to a manifestation of this kind, observed in 1938 in a small village of the Lambayeque Department, situated in a mountainous area 1300m above sea-level. There an epizootic among R. rattus led to the appearance of the disease in guinea-pigs kept in the house of the

*According to the PAHO/WHO Scientific Publication No. 48 ("Recorded cases of notifiable diseases in the Americas 1949-1958", Washington 1960), three cases were recorded in 1958 in the Tumbes department, the case incidence for the above-mentioned departments being Piura-31, Lambayeque-7, Cajamarca-3 and Ancash-5.

first victim. Two children in a neighboring house to which some garments of this woman had been brought, also contracted the infection but recovered under serum treatment. In the opinion of Ramos Díaz, X. cheopis, the principal flea of the rats in the locality, had been responsible for the infection of the domesticated guinea-pigs, but P. irritans had been instrumental in conveying P. pestis from the latter to the first victim. He claimed to have proved the presence of this organism in a lot of P. irritans collected from a garment used by the second affected family, but the technique he used for this purpose can not be considered as fully reliable. Be this as it may, it is not easy to believe that in a locality where rats infested with X. cheopis had become plague-affected, this vector had not been responsible for the human infections.

As has been noted above, the presence of plague in the Lambayeque Department was again recorded in 1958, but it would seem that on this occasion a coastal and not a mountainous area was affected.

Describing a successful anti-plague campaign with DDT and "1080" (sodium fluoro-acetate) in Tumbes, the capital of the department of the same name in 1945, Machiavello stated that before the outbreak fought by him this town had suffered from the disease in 1909, 1915, 1922 and 1940. In his opinion the cause of the 1945 outbreak had been the importation of fleas in bags of merchandise from the Lambayeque Department. 95% of the rats then present in Tumbes were R.r. alexandrinus, 5% R. rattus, all of which were almost exclusively infested with X. cheopis.

As noted above, the presence of plague in the Tumbes Department was again recorded in 1958.

Manifestations of wild rodent plague.

1) Trujillo area

In reference to the situation in the area of Trujillo, capital of the department La Libertad, Macchiavello (1958) made the following statement:

> "During the plague control campaign in Trujillo we could repeatedly confirm a re-infection derived from the surrounding rural area where an epizootic is maintained in the sigmodons (S. peruanus). Suddently, during a season unfavorable for the activity of X. cheopis, plague attains epizootic proportions in the rural area, where every day hundreds of Sigmodons succumbed to the infection are found. R. r. frugivorus which lives together with Sigmodon in the fields and breeds particularly round the small hamlets, was practically not touched by the epizootic during the cold season, in which X. cheopis remains inactive and in which the transmissions from one species to the other depend upon P. litargus.....The epizootic becomes first manifest in R. frugivorus and afterwards passes to R. rattus and R. alexandrinus of the settlements only when the season becomes favorable for X. cheopis and the infection originally acquired by a few rats in the fields can spread among these rodents. During the summer the epizootics in

these two rat species follow an independent course and the number of X. cheopis on the Sigmodons was not more important than that of P. litargus recuperated from the rats".

It would thus seem that, while S. peruanus had become the reservoir of plague, common rats and X. cheopis were responsible for its spread in the settlements.

2) Lancones district

In his article on plague in the Lancones district (Sullana Province, Department of Piura), to which further reference will be made below, Macchiavello(1957) furnished the following data on the early rat-caused and cheopis-borne bubonic outbreaks in the provinces of Paita and Piura:

	Paita		Piura		Sullana		
Year	City	Province	City	Province	City	Province	Lancones district
1904	174	174	0	0	0	0	0
1905	80	80	0	0	0	0	0
1906	59	59	0	26	0	0	0
1907	177	177	27	90	0	0	0
1908	29	39	19	140	1	1	0
1909	46	46	6	23	0	0	0
1910	0	0	12	49	0	0	0
1911	1	1	13	92	0	0	0
1912	29	29	8	176	1	1	0
1913	15	15	7	9	0	0	0
1914	0	0	40	94	0	0	0
1915	0	0	25	52	0	0	0
1916	50	54	0	21	3	3	0
1917	0	0	1	14	0	0	0
1918	17	17	55	93	61	61	0
1919	101	102	9	22	4	4	0?
1920	63	63	24	52	33	33	0
1921	68	84	8	104	17	17	0?
1922	39	42	20	25	9	9	0
1923	31	31	32	47	1	1	0
1924	12	12	0	0	2	2	0
1925-27	0	0	-	-	0	0	0
1928	3	3			0	0	0
1929	13	13			0	0	0
1930	-	-			0	0	0

As indicated by this table, plague showed little tendency in the province of Paita to spread to the hinterland, remaining practically restricted to the port of Paita, while in Piura Province the incidence of the disease in the capital was often considerably below that in other localities of the province. As far as can be judged from the available data, plague ceased to be manifest in the latter province in 1925, in Paita in the year of 1930.

Macchiavello felt certain that the appearance of plague in the city of Sullana, where the disease was quite active from 1918 to 1924, was due

to an invasion from the two above-mentioned provinces. There can be hardly any doubt that this entry of the disease eventually led to an entrenchment of plague in the wild rodent population of the Lancones district and also of an adjacent Ecuadorian area which, though becoming manifest only in 1946, presumably commenced considerably earlier, perhaps already during the period from 1919 to 1921. Macchiavello noted in this connexion that one plague case had been recorded in 1939 at Huasimal in the Lancones district and that possibly three human attacks occurred there in 1943. The reappearance of the disease in June, 1946, in the Lancones district and the simultaneous involvement of the adjacent Cazaderos-Alamor area in Ecuador led to an investigation under Macchiavello, the main results were as follows:

a) Domestic rodents and their fleas - The common rats were entirely absent in the affected Peruvian area. Their existence in Bolasbamba, one of the affected localities on the Ecuadorian side, was reported, but could not be confirmed. Mus musculus were present in most of the houses but with the exception of one animal trapped in the house in habited by the study group, on which one X. cheopis was found, the not numerous specimens seen were free from fleas. Macchiavello felt certain that the cheopis had been imported in the baggage of the detachment.

b) Wild rodents - The species of wild rodents met with and the occurrence of plague in them is shown in the following table:

Species met with Sciuridae :	Number Examined	Found plague-infected
Sciurus stramineus nebouxi	51	14
Cricetinae:		
Oryzomys xantheolus xantheolus	18	3
Oryzomys nitidus	5	0
O. stolzmanni stolzmanni (O. longicaudatus auctt.)	1	0
Rhipidomys equatoris	11	3
Akodon mollis mollis	3	1*
Akodon mollis orophilus	1	0
Sigmodon simonsi	1	0

* Found positive in Ecuador

Dealing with the findings in the squirrels, Macchiavello stated that (a) only two or three of his positive specimens showed macroscopic signs of rather subacute plague, associated with the presence of numerous P. pestis in their organs; (b) on the other hand guinea-pig experiments made with the organs of apparently healthy squirrels gave positive results; and (c) the plague strains isolated from some of the squirrels showed an attenuated virulence which, however, could be restored through guinea-pig passages. He concluded from these findings that besides an active form of plague a 'residual' type of the infection was apt to occur in the squirrels.

It is important to add that in many of the examined squirrels organisms of the aspect of P. pestis were seen in smears but guinea-pig tests made with material from such animals gave a negative result.

In marked contrast to the findings recorded above, most of the positive cricetinae showed signs of acute plague with numerous P. pestis in the smears. Commenting on the rarity of positive results in these animals, Macchiavello stated -

> "The relatively low incidence of plague in the cricetidae stands in contrast with the relatively high occurrence of inapparent or resolving plague met with in sciurus. This may be due to the fact that the cricetidae are more susceptible to the infection and succumb to it, being almost immediately devoured by birds of prey".

c) Wild-rodent fleas - The fleas met with on the wild rodents all belonged to the genus Polygenis, most of them being identified as P. litargus, while some, found on Akodon mollis mollis in the Ecuadorian part of the focus, were of another still unidentified species. The flea index of the cricetidae was fairly high (2.4 in the case of O. xanthaeolus, 1.1 in that of Rh. equatoris and 3 in A. mollis), even though most of the animals had been caught with the aid of cyanogas. The flea index of the squirrels, on the contrary, was only 0.22, solely 16% of these rodents being flea-infested. Commenting on this observation, Macchiavello stated: "in general the wild-rodent fleas are more sedentary than the rat-fleas and prefer the environment of the homogeneous temperature of the nests or of dark and cool places. The observation that in May (a month with moderate temperature in Lancones) the rodents harbor many fleas and of the occurrence of comparatively many free fleas on the harvested maize fields .... makes it probable that at a certain season of the year the flea index of the rodents is high and that, undoubtedly, these free fleas come from rodents succumbed at that time to plague. This holds true of the squirrels as well as of the cricetidae in general".

The presence of plague was confirmed with the aid of pooling tests in (a) a batch of 3 P. litargus collected from squirrels, and (b) a lot of 14 fleas of this species, which had been kept under observation in a hollow tree for 6 months. Thus P. litargus, besides conveying the infection, was evidently also capable of maintaining it.

Referring to his successful vector studies with P. litargus, Macchiavello stressed the willingness and capability of this flea to feed on man.

d) Epidemiological observations - As can be gathered from Macchiavello's article, during the 1946 outbreak observed by him, a total of 28 attacks of bubonic plague with 6 deaths was recorded, 20 in persons living in 10 different localities of the Lancones district and 8 in people inhabiting 4 localities of the Cazaderos-Alamor area in Ecuador. As

he emphasized, "if not all, so the majority of the cases stood in relation with previous activities in the maize-fields. Some fell ill also staying on maize farms. With rare exceptions, all the individuals slept in places where some of them noted an abundance of fleas. At least 6 of the patients observed dead rodents in the maize-fields or adjacent fields or found evidence of epizootics among them".

Evaluating these observations and the findings he had made in the rodents and their fleas, Macchiavello came to the conclusion that plague in the Lancones District and the adjacent areas of Ecuador was of a purely sylvatic type, a presistence of the infection in the squirrels leading periodically to epizootics among the cricetinae which in their turn were mainly responsible for the appearance of human outbreaks. Polygenis fleas, in the first line P. litargus served in all these manifestations as the vector of the infection.

3) Huancabamba and Ayabaca provinces

Whereas no recent information on the Lancones district is available, the early studies on the plague situation in the provinces of Huancabamba and Ayabaca by Macchiavello in 1946-1947 have been followed by investigations made in 1955-1956 and again in 1960 by De la Barrera who, however, mostly reported on observations in the first mentioned province (Huancabamba).

In his report, Macchiavello furnished the following general information on the two provinces:

	Huancabamba Province		Ayabaca Province	
Area (km^2)	6557		7082	
Population (1940 census)	51613		75068	
		Inhabitants		Inhabitants
Capital	Huancabamba	2580	Ayabaca	2620
Districts	Huancabamba	21605	Ayabaca	24255
	Canchaque	8540	Cumbicus	13350
	Huamarca	13242	Frías	18122
	Sondor	3027	Montero	5777
	Sondorillo	2462	Sicches	2856
			Suyo	6727

The population of these areas consisted almost exclusively of Indians or mestizos, whose standard of life was deplorably low. The climate, and accordingly the vegetation, varied in relation to the altitute of the different regions, with forests in the low-lying valleys (in some of which tobacco and cocoa were cultivated), scanty growth in

the high regions, cultivations of cereals and alfalfa in the prairie regions and on the slopes of the hills. The plague area of Huancabamba was mainly wheat-growing, groups of workers migrating from one cultivated locality to the others to perform the necessary labors and thus while sojourning in the fields, being under the risk of infection, if plague epizootics were present. Of great epidemiological importance was also the practice of the people to store the harvested wheat in their houses, especially in the attics with which many of the houses were provided. Thus, the grain stores in the houses attracting the wild rodents, at the time of epizootics facilities were created for a transition of infected fleas to the intra-domestic rodent fauna or directly to man, especially if wild rodents succumbed to plague in the storerooms.

The intradomestic rodent fauna consisted of M. musculus (which, however, sometimes lived in contact with the cricetinae in the fields round the houses) and of the amply bred guinea-pigs. However, Macchiavello insisted, except in the regions at the foot of the mountains, Rattus was absent.

The prevalent domestic flea, P. irritans, occurred not only - often in almost incredible numbers - in the clothes, on the earthen floors, etc. but also infested the domesticated guinea-pigs, sometimes to a higher degree than their specific flea, Tiamastus cavicola. P. irritans was likewise found on the house-mice on which, however, it was outnumbered by their specific flea, Leptopsylla segnis.

Macchiavello found in the course of his work only one specimen of X. cheopis in the Changra region, a zone situated at low altitude in the border district between the Ayabaca and Morropón provinces in which the plague incidence during the 1946-47 epidemic was unusually high. Generally speaking he upheld that this flea might be present in the regions at the foot of the mountains. This seemed to hold true for instance of the low-lying Chalaco district in the province of Morropón, the involvement of which in the 1946-47 outbreak presumably stood in causal relation to the presence of R. rattus and X. cheopis.

Referring only to the wild rodents and lagomorpha involved in the 1946-47 outbreak, Macchiavello enumerated the following species:

a) Akodon mollis orophilus, the most prevalent species, often and sometimes to a high degree infesting the houses, particularly at the time the grain supplies were stored in them and apt temporarily to occupy the nests of M. musculus (which thus could become infested with wild rodent fleas)*;

b) An undetermined species of Oryzomys;

*In localities where no cereals were cultivated, Akodon mollis oroph. was apt to live round the houses, particularly in the stone-walls separating the various properties.

c) Oryzomys stolzamanni stolzamanni (O. longicaudatus stolzamanni auctt.) also a widely distributed species;

d) Cavia tschudii ssp., a species distinct from the usually domesticated Cavia porcellus and, in contrast to the latter, apt to lead a peridomestic or even 'sylvatic' existence;

e) Sylvilagus species, presumably S. andinus and S. ecaudatus.

The rather scanty data supplied in the study presently under review in regard to the wild-rodent fleas may thus be summarized:

Number and species of rodents searched	Number of fleas fd.	Species of fleas
33 A. mollis orophilus	20	Besides 3 L. segnis Pleochaetis dolens quitanus, P. equatoris, Polygenis litargus and Plocopsylla mars.
8 O. stolzamanni	8	1 Polygenis litargus and 7 Pleochaetis, mostly P. dolens quitanus
16 cavia tschudii (mostly from houses)	997	P. irritans, L. segnis, T. cavicola, Ct. felis, H. suarezi, Pl. dolens quitanus and the specific Rhopalopsyllus cacicus
4 Sylvilagus sp.	118	Pl. dolens quitanus (4), Pol. litargus (3), Pol brachinus (33), R. cacicus (27), Hoplopsyllus manconis (11), Cediopsylla spyllmanni (40)

NB It is important to note that Sylvilagus and probably also O. stolzamanni were infested only with wild rodent fleas.

Dealing with the history of plague in the two provinces under review, Macchiavello maintained that the disease first appeared in 1920 in Huancabamba

Province at Canchaque, situated halfway between Piura and Huancabamba City, a locality up to the present infested with R. rattus and X. cheopis. The involvement of the city of Huancabamba in 1923 led in its turn to a spread of the infection to the rural areas. From 1928 plague in the capital became sporadic but continued to become manifest in more and more distant villages. In Ayabaca Province the disease was first recorded in 1922, as far as it is known initially in a hamlet near the capital, and also spread in the rural localities.

The affection of both provinces was presumably due to the importation of infected cheopis fleas from the coastal areas in goods or in the baggage of travellers, the presence of a numerous and susceptible wild rodent population offering ample fuel for the spread of disease. To judge from data furnished by Macchiavello human plague in the foci of Huancabamba and Ayabaca, which became eventually confluent, showed a seasonal incidence, being manifest in spring and summer, but absent in winter.

Dealing with the types of plague observed in Huancabamba Province, Macchiavello furnished the following global statistics:

	Number of patients						Total		
	1939-1945			1946-1947			1939-1947		
Type of plague	Men	Women	Total	Men	Women	Total	Men	Women	Total
Without data			23			7			30
Septicemic	2	1	3	5	11	16	7	12	19
Bubonic	57	44	101	123	157	280	180	201	381
Totals			127			303			430

As far as these data go, the overwhelming number of the patients whose histories were known, suffered from bubonic attacks and pneumonic plague was altogether absent. However, Macchiavello referred to vague reports speaking of whole families succumbing to the disease which were suggestive of the latter type of the plague. It appeared that under such circumstances the neighbors were prone to burn down the affected huts without even making sure that all inmates had died.

Comparing the earlier epidemics with the 1946-47 outbreak, Macchiavello stated -

> "Before 1946, plague appeared in isolated houses and the number of human cases did not stand in direct relation with the plague in the wild rodents, the intensity of which was not known. In 1946-47, the intensity of the wild rodent epizootic could be guessed, not only through the rapid and successive infection of 50 odd villages but also through the extension of the area in which it was observed. In each hamlet plague acquired a familial form and it is this appearance of the epidemic which favors the false supposition of an interhuman transmission*, which, moreover,

*Macchiavello referred in this connexion to an unpublished article on the interhuman transmission of plague and also said in the above reviewed report that "if P. irritans were a plague vector, the human population would have disappeared from the Andean territory".

is favored by the frequent observation that secondary foci appeared in new hamlets precisely in the houses inhabited by members of plague-affected families who had flown from the infection in their own villages".

It would also seem that the mortality in the 1946-47 epidemic (52.8%) was markedly higher than that during the period from 1939-1946, when 23 out of the 80 patients, about whom details are known, seem to have succumbed to plague.

Referring to his observations on plague manifestations in the rodents, Macchiavello stated to have proved the presence of the infection in 15 or 16 out of a total of 124 specimens, namely in one out of 63 house-mice, in 6 out of 33 Akodon mollis, in one (or possibly in 2) out of 8 O. stolzmanni, in 4 out of 16 Cavia tschudii and in 3 out of 4 Sylvilagus. In the opinion of this observer the appearance of the infection not only in Oryzomys and C. tschudii, but also in Sylvilagus was of a secondary nature. In regard to the last mentioned genus he noted -

"References to epizootics among the rabbits in the rural (agrestes) areas and at the foot of the mountains were frequent. The tardy appearance of these epizootics and the fact of finding on the rabbits Akodon fleas indicate that the epizootics were secondary, possibly originating in the deserted wheat fields, where an enormous infestation of Akodon fleas persisted in the empty nests and where, besides this, occasionally rabbit fleas were found. These, however, were not found on the akodons captured in houses".

In regard to the role of the wild-rodent fleas Macchiavello stated that, following up his earlier studies on P. litargus in the Trujillo area and the Lancones district he (a) established the vector capacity of Pleochaetis dolens quitanus and, moreover, found the following fleas naturally plague-infected: Cediopsylla spillmanni, Hoplopsyllus andensis, H. manconis, Polygenis branchinus and Sphinctopsylla (Plocopsylla) mars. Positive results were also obtained with a pooled sample containing among other fleas Rhopalopsyllus cacicus cacicus. The majority of the positive fleas had been collected from plague-affected rodents, but infected Pleochaetis were found also in deserted wild-rodent nests, P. brachinus once in a 'domestic' nest.

Commenting on these findings, Macchiavello stated -

"The comparative importance of the 2 Pleochaetis species and the 3 Polygenis species met with in the transmission of plague among the cricetinae and from these to man, remains to be determined. Judging simply by their numerical frequency, one may infer the preponderant role of Pleochaetis dolens quitanus."

Exact studies were also required to delimit the habitat of the various flea species.

In Macchiavello's opinion P. dolens quitanus was responsible for the conveyance of plague to man in the houses as well as in the open, even though this flea seemed by preference a nest-dweller. Its mean

extrinsic incubation period was about 25 days in the laboratory, but was probably much longer in the open at high altitudes, where from August to November the nights were rather cold.

Summarizing and evaluating his observations on the 1946-47 outbreak, Macchiavello distinguished between (a) an initial phase in which the evolvement of a violent epizootic among the cricetinae, especially Akodon mollis orophilus, led to occasional infections of field workers, conveyed by P. dolens quitanus and P. litargus, especially the first; (b) a main phase, in which the wild rodents, attracted by the stores of the harvested cereals in the houses brought the infection to the villages, an epidemic of a 'familial domestic' character ensuing; (c) a final phase in which Sylvilagus, leading by preference a sylvatic existence and free-living guinea-pigs (C. tschudii) became the victims of not properly studied epizootics, in which probably Hoplopsyllus and Polygenis fleas acted as vectors of the infection.

Factors favoring the spread of infection at the acme of the outbreak were (i) panicky flight of the people; (ii) removal of domesticated guinea-pigs to other settlements so as to avoid their destruction by the plague prevention staff; and (iii) involvement of the peridomestic wild rodent species, particularly Oryzomys sp. and Oryzomys stolzmanni, in the epizootics.

It is important to add that in Huancabamba Province the spread of plague in man was cut short by the ample use of DDT. However, wild rodent plague continued in enzootic form, leading especially in the formerly infected localities to sporadic manifestations in man. In Ayabaca, where less systematic use of DDT had been made, plague showed some tendency for further progress, reaching in 1953 the districts of Montero, Suyo and Sicches, adjacent to Ecuador.

The statements of Macchiavello will be evaluated together with the more recent findings of De la Barrera summarized below:

In marked contrast to the above recorded observations, De la Barrera stated in his 1957 report that R. rattus was found side by side with the ubiquitous house-mice and, usually, domesticated guinea-pigs not only in the houses of Huancabamba city, but also in the rural dwellings of that area, in which, however, it was rare. He found no evidence that the common rats were involved in the plague outbreaks and explained the absence of the disease among them by the assumption that they were resistant to infection with P. pestis. According to De la Barrera's observations the wild rodent fauna of Huancabamba Province was identical with that generally met with in the plague regions of Peru. Akodon mollis formed 85% of the rodents trapped in the fields, Oryzomys xanthaeolus being accordingly less frequent. 4 rodents of the former species and one of the latter were caught inside rural habitations. Plague was confirmed in 1956 in two specimens only - once in A. mollis and one time in 'Oryzomys longicaudatus'.

Dealing with the wild rodents and lagomorpha of the plague-affected areas of Peru in general, De la Barrera specially referred to the following species:

a) A. mollis and O. xanthaeolus were the species most often met with and most often found plague-infected. Their relative frequency varied in different sites, but generally the first mentioned species was prevalent at higher altitudes.

b) Sigmodon peruanus was frequently found plague-infected but, as De la Barrera somewhat ambiguously stated "on account of its much lesser abundance than that of Oryzomys and of Akodon, does not appear to figure on the first level in the list of plague reservoirs". S. peruanus was a slow-moving animal, which left its dens during daytime. It constructed its nests on the level of the ground under various protecting coverages, but, if disturbed by man, was apt to hide in cracks of the ground. As far as scanty observations went, it was almost exclusively infested with P. litargus.

c) Proechimys decumanus, a large rodent met with only in the northwest of Peru near the Ecuadorian frontier. It was always heavily infested with Polygenis klagesi, but was also found to harbor some P. litargus.

d) Sylvilagus brasiliensis inca, the rabbit (conejo) of Peru, was also found plague-infected. However, in De la Barrera's opinion, its affection was "an accident in the progress of the wild-rodent epizootics". The specific flea of this species of lagomorpha was apparently Hoplopsyllus manconis.

Dealing with the flea fauna of the rodents in Huancabamba city and its environs, De la Barrera furnished the following important data:

Species of fleas	City		Rural houses		Fields		Wild rodents		Rodent nests	
	No.	%	No.	%	No.	%	No.	%	No.	%
Xenopsylla cheopis	52	53.6	31	20.9	18	43.9	-		-	
Pulex irritans	38	39.1	27	18.2	3	7.3	1	0.4	1	1.0
Leptopsylla segnis	4	4.1	60	40.5	-		1	0.4	-	
Ct. felis felis	2	2.2	5	3.3	-		2	0.8	-	
E. gallinacea	1	1.0	11	7.0	-		-		-	
Pleochaetis dolens quitanus	-		7	4.7	10	24.3	44	20.5	10	11.7
Craneopsylla minerva	-		7	4.7	6	14.6	42	19.2	-	
Polygenis brachinus	-		-		4	9.7	95	44.3	55	64.7

(NB See over.)

NB. Found only on wild rodents or also in the rodent nests were: Neotyphloceras rosenbergi, Ctenidiosomus spillmanni, Plocopsylla hector, Hoplopsyllus manconis, Plocopsylla n.sp. and Cleopsylla townsendi.

The most significant among these findings are (a) the infestation of the common rats in all locations with X. cheopis; (b) the comparatively frequent occurrence of P. irritans on this rodent species; and (c) the presence of not inconsiderable numbers of wild rodent fleas on the rural rats.

In the part of his report devoted to a discussion the Peruvian rodent-fleas in general, De la Barrera made the following statements:

a) X. cheopis - Generally speaking this flea was more numerous on the settlement-rats than on those trapped in the fields. Its frequency decreased with the altitude, so that, while still met with in the Huancabamba region (1900-2000m), it was absent in the higher parts of the province, e.g. at Silur (2740m) and Jacocha (2840m). Which flea replaced it there, is obviously an unsettled problem. The only fact known in this respect seems to be that De la Barrera found only one specimen of Nosopsyllus londinensis in his flea material.

In the opinion of this observer, X. cheopis took no part in the spread of the infection in the Peruvian sylvatic plague foci. He maintained in this connection that possibly in the Huancabamba area and other localities situated near the upper limits of its habitat the vector capacity of this flea was reduced but admitted that this assumption did not hold true for other localities like for instance Llampa* and Puná in Ecuador, where conditions for a role of this flea seemed to be excellent. De la Barrera evidently inclined to the belief that the failure of X. cheopis to convey the infection stood in relation to the resistance of its rat-hosts to plague.

b) Pulex irritans was found in great quantities in the garments and bedclothes of the inhabitants and, as noted above, was by no means rare on the common rats. Of course it was also frequent and sometimes prevalent on the domesticated guinea-pigs.

Discussing the possible importance of this flea in the spread of plague, De la Barrera drew attention to the contention of Karl Jordan and F.G.A.M. Smit that P. irritans was not a homogeneous species but consisted of several forms. Referring with the two varieties met with in the Peruvian material, Smit stated -

"The new Pulex is different from the cosmopolitan human flea, Pulex irritans L.....it may well be that the rodent irritans could also act as a carrier of the wild rodent plague".

* As recorded by De la Barrera, an epizootic involving mainly Oryzomys xantheolus in the region of Llampa in 1955 led to six plague attacks in man. R. norvegicus, though frequent in that locality in and round the houses, remained free from the infection.

Reverting to this problem in a later part of his report, De la Barrera cautiously noted -

> "The possibility that this flea could be a (plague) vector, specially in interhuman infection, has attracted attention since long ago and in this part of America has been given as an explanation for the frequent infections among the participants in wakes which, of course, is purely theoretical. Of different value are the experimental data of Burroughs, that P. irritans becomes blocked and can, as a consequence, transmit (plague). Mention has been made also of the observations made in the British Museum regarding the heterogenicity of Pulex irritans..."

There can be no doubt that the recent observations of Jordan and Smit necessitate a reconsideration of the possible role of P. irritans in the spread of plague - a problem which to great advantage could be studied in the course of the proposed plague investigations in South America.

c) Tiamastus cavicola was frequent and often prevalent in Peru on the domesticated guinea-pigs and occasionally was also found on the domestic mice. De la Barrera thought that this flea, though not a good vector, might play a role in the intradomestic spread of plague.

d) Wild-rodent fleas - According to De la Barrera's observations, Polygenis litargus was not only the most frequent flea on the wild rodents, but was also met with on R. rattus living under rural conditions as well as on the domesticated guinea-pigs and in small numbers in garments. Though not yet aware of the studies on the vector capacity of this flea, De la Barrera admitted that it might play a role in the conveyance of plague even to man. He reiterated, however, that certainly it was not the only plague vector, as it was absent in the sylvatic plague areas situated above about 2200 m. where its place was taken apparently by Pleochaetis dolens quitanus.

Referring briefly to the 1956 plague outbreak occurring in numerous foci lying at high altitude in Huancabamba Province, De la Barrera summarized that the epidemic, being preceded by a wild-rodent epizootic, remained restricted to rural areas, neither the rats nor the human population of Huancabamba city becoming involved. Instances of an appearance of successive attacks in one and the same household were evidently frequent. De la Barrera considered it as significant that (i) in all houses with multiple attacks one or more of the first affected patients died, no doubt after they had developed a generalized infection; and (ii) P. irritans abounded in all the dwellings.

According to De la Barrera's final report, the plague outbreak in the province of Huancabamba in 1960* was also characterized by the

*As stated in this report during the first 5-1/2 months of 1960 a total of 101 plague cases was recorded in Peru, distributed in the following provinces: Huancabamba (77), Ayabaca (15), Hualgayoc (7), and Canchaqui (2). The total mortality was 36%.

frequent occurrence of several attacks in the affected houses, in which often infection of the domesticated guinea-pigs preceded the appearance of human infections. Which species of fleas were responsible for the conveyance of the disease from these animals to man, was not clear.

As summarized in the 1960 report, the salient features of this as well as of the previous recent outbreaks were:

i) A sylvatic origin;
ii) Absence of the infection from the centers of the population;
iii) Seasonal incidence during the rainy period (i.e. usually from November to April or May);
iv) Frequency of multiple attacks in one and the same house;
v) High mortality due to frequent lack of treatment.

Evaluation and recommendations for further investigations.

The task of evaluating the present plague situation in Peru is fraught with great difficulties in so far as fairly up-to-date information, comparable to that available for Venezuela and Ecuador, could be obtained only for the sylvatic plague focus in the provinces of Huancabamba and Ayabaca. To what extent plague is still active in the Lancones district formerly studied by Macchiavello, could not be ascertained, while for the other recently affected parts of Peru really nothing is known than the bare figures recording the appearance or recurrence of the disease. Hence, should it be considered advisable to make plans for plague investigations in Peru on a countrywide scale, it would be indispensable to establish through consultations with the Plague Prevention Service and, if necessary, through a preliminary survey to what extent it would indicate to envisage work in the last mentioned areas. If work in the Lancones District would be called for, it ought to be done according to the plan set forth in sufficient detail in the preceding section of this report for the adjacent areas in Ecuador. Indeed, since these and the Lancones District are but component parts of one common plague focus, work in these parts could be profitably done only by an international team having free access to the plague areas situated south and north of the border between the two countries.

In view of this and since there can be hardly any doubt that, even if a countrywide plan for plague investigations in Peru would be decided upon, priority would have to be given to the most seriously affected Huancabamba-Ayabaca focus, it seems legitimate to concentrate for the present attention upon this area.

While the earlier investigations made in this focus leave no room for doubt that it is one of sylvatic plague, the question which species of wild rodents is or are the reservoir of the infection, does not yet seem to have been satisfactorily answered. As has been noted, in contrast to the conclusions reached in other South American sylvatic plague foci Macchiavello maintained that in the Huancabamba-Ayabaca area the infection is permanently harbored by species of cricetinae which, because apparently highly and uniformly susceptible to plague, are periodically decimated by wide-spread epizootics. Thus, in the opinion of this observer, the other species of wild rodents found plague-infected, including

Sylvilagus, which has been incriminated as reservoir of the infection in Ecuador, play only a secondary role or, as maintained by De la Barrera, become in part only accidentally involved. The present reporters do not wish categorically to refute these postulations. They must insist, however, that the scope of the observations upon which they were based, is really insufficient for a final decision of the problem. Thus, in order to solve it, further large-scale and systematic observations, made not only at the rather impropitious time of the plague seasons but continued throughout at least one year, preferably for a longer time, are indispensable to ascertain where and in what species plague persists during the interepizootic periods. It deserves great attention in this respect that, as mentioned by Macchiavello, apparently these strongholds of the infection are limited in their number and their extent. To prove the existence of such 'elementary' foci and thoroughly to study their ecology would be not only scientifically valuable but of great practical importance.

The question which fleas are responsible for the spread of sylvatic plague in the Huancabamba-Ayabaca focus is also by no means settled. While several species have been found naturally infected, experimental studies to assess their vector capacity have been made only in the case of Polygenis litargus and Pleochaetis dolens quitanus. To confirm the results of these tests, to determine the vector capacity of at least the flea species which have been or will be found naturally infected and thoroughly to study the distribution of the various species, would be a second task indispensable for a thorough knowledge of the ecology of the disease in the focus.

While Macchiavello found in the past the common rats to be present only in the low-lying valleys of the Huancabamba-Ayabaca area, De la Barrera's more recent experiences indicate the presence of these rodents in all parts of the focus, though rarely in the rural habitations. At the same time the latter observer insisted that the rats, because resistent to infection with P. pestis, were not involved in the plague manifestations. To judge from the published information, however, it would seem that the postulated resistence of these rodents has not been confirmed through laboratory tests. To fill this gap hand in hand with systematic investigations on the distribution of the common rats in the focus, would be a third important object of the proposed studies.

A further unsettled problem is which flea replaces X. cheopis on the rats in the highly situated parts of the focus. Besides adequate studies to settle this point, systematic investigations would be called for to study the frequency of X. cheopis in the rural areas as well as in the towns within the zone of its habitat. Due attention ought to be paid in the course of these investigations to the occurrence of this flea on hosts other than the common rats, particularly the domesticated guinea-pigs. If found to be rare or absent on the rodent species other than the rats, tests ought to be made to assess the capability of X. cheopis to subsist and feed upon the domesticated guinea-pigs and on other rodents it might have an opportunity to attack. The assumption of De la Barrera that in localities near the upper limits of its habitat X. cheopis is a less efficient plague vector than usually is an interesting one. However, it would be rather difficult to test the validity of this postulation.

To judge from the hitherto available information, the mechanism by which the infection originating in the wild rodents of the Huancabamba-Ayabaca plague focus ultimately finds its way to man, has not been fully elucidated. This transition of the infection could be satisfactorily explained in the past when, following the pattern of 'purely' sylvatic plague, the sporadic human affections were mainly if not solely contracted in the fields. However, attention had to be drawn to the fact that from 1946-47 onwards an intradomestic type of infection was the rule, often resulting in the appearance of not one but some successive attacks in one and the same house. There can be little doubt that an affection of domesticated guinea-pigs, due to the bites of infected wild-rodent fleas, was often a link in the chain of factors leading to the appearance of plague in man. However, it is still quite uncertain, which flea species are responsible for the human infections. Attention has been drawn in this connexion to the possibility that the appearance of successive plague attacks in one and the same household might be the result of an intrahuman spread of the infection conveyed by P. irritans. As mentioned earlier in this report, the recent discovery that this species is not homogeneous has led to the suspicion that some of the subspecies composing it might be efficient plague vectors. It would be essential, therefore, to make a thorough comparative study of the vector efficiency of the subspecies of this flea met with the Huancabamba-Ayabaca focus. At the same time, however, systematic studies of the intradomestic and peridomestic flea-fauna in general are called for, paying special attention to the houses and settlements recently or presently attacked by plague.

A close study of the manifestations of plague in man would have to form an integral part of the proposed investigations. The records on the incidence of the disease during recent years would have to be carefully gone through so as to detect localities in which plague has appeared repeatedly or even perennially and every possible effort would have to be made to elucidate the reasons for such a persistence of the infection. As proposed already in the report on Ecuador, all human plague manifestations met with by the study group ought to be made the subject of thorough investigations. In the first line efforts should be made to establish which rodents and which fleas were responsible for the infection of the patients. The presence of plague in the latter ought to be confirmed invariably through adequate laboratory tests and the strains of P. pestis isolated from the sufferers ought to be kept in a lyophilised condition for further study. Moreover efforts ought to be made to detect the occurrence of subclinical forms of the disease through clinical and serological surveys of groups of the population actually or potentially under the risk of infection.

In his reports De la Barrera justly and laudably stresses the necessity of improving the case-finding system so as to reduce the deplorably high mortality from plague in patients who, because detected not timely enough or even not at all, could not be given the benefits of treatment. While thoroughly concurring with this plea, the present reporters wish to emphasize the necessity of going one step further by trying to detect not the early incidence but the imminence of human plague through a constant watch over the fluctuations of the density of the rodent populations and

the frequency of plague manifestations in them. Every possible effort ought to be made therefore in the course of the proposed studies to work out such a system of gauging the plague situation in the Huancabamba-Ayabaca focus. For in this way the contemplated work would not only contribute materially to a knowledge of the ecology and epidemiology of plague in South America and possibly pave the way for future attempts to control the situation but would be of immediate benefit to the people exposed to the risk of infection.

SECTION F

BRIEF OBSERVATIONS ON THE INCIDENCE AND ETIOLOGY OF PLAGUE IN THE UNITED STATES

The incidence of human plague in the United States from the first recorded appearance of the disease in the port of San Francisco in 1900 up to 1961 is summarized in the following tabulation:

RAT CAUSED EPIDEMICS OF PLAGUE IN U.S.A.

	Year	Cases	Deaths
San Francisco, California	1900-4	120	114
	1907-8	186	92
Seattle, Washington	1907	3	3
New Orleans, Louisiana	1914-15	31	10
	1919-21	25	11
Pensacola, Florida	1920	10	4
Galveston, Texas	1920	18	12
Beaumont, Texas	1920	14	6
Los Angeles, California	1924	41	34
Total		448	286

INFECTION CONTRACTED FROM WILD RODENTS, 1908-1961

	Human Plague	Cases	Deaths	Infected Rodents found
California	1908-61	56	52	1908
Oregon	1934	1	1	1935
Utah	1936	1	0	1936
Nevada	1936	1	0	1936
Idaho	1940	1	1	1936
New Mexico	1949	12	5	1958 (1903)
Colorado (Texas)	1957	1	1	1941
Arizona	1950	1	0	1938
	Total	74	60	

U.S.A. total cases 532; 346 (65%) deaths

A tabulation showing the annual incidence of plague in California is appended to this report (see Appendix 1).

At a first glance these data seem to indicate that the evolution of the plague in the United States was in accordance with the pattern typical for several other countries invaded by the sea-route during the present pandemic, an initial 'murine' phase of the infection eventually leading to a spread of the disease to the wild-rodent populations. However, in the opinion of some observers the appearance of plague in North America was due not to its recent introduction by sea, but to an early importation through wild rodents which, coming overland across the land bridge later replaced by the Bering Strait from Central Asia, brought P. pestis with them as a population regulator. This fascinating hypothesis was rejected by other authors, mainly on the ground that, in contrast to the plague strains isolated from the Central Asiatic wild rodents, those from both the rats and the wild rodents in America failed to produce an acid reaction in glycerol-containing media. At the same time some of the opponents to the above-mentioned hypothesis considered it as likely that plague existed in California before its appearance was officially recorded in 1900, the infection having been imported from the China coast (affected since 1867) at an earlier date. The same apparently holds true of the sylvatic manifestations, wild-rodent plague, though definitely detected in California in 1908, having evidently existed in the Contra Costa country of that state since 1903 at least.

While, as shown by the above-inserted table, rat-caused epidemics ceased to occur after 1924, the infection among the wild rodents persisted and spread so that according to statistics of Link quoted by Pollitzer by 1946 besides California 14 other states had become involved thus:

State	Year of Detection	State	Year of Detection
California*	1908	Arizona*	1938
Montana	1935	New Mexico*	1938
Oregon*	1935	Colorado*	1941
Idaho*	1936	North Dakota	1941
Nevada*	1936	Oklahoma	1944
Utah*	1936	Kansas	1945
Wyoming	1936	Texas	1946
Washington	1937		

*Human plague

Fortunately, as indicated in this table and in that inserted above, the presence of wild-rodent plague led to mostly rare human infections in only eight of these states.

As shown in two tables attached to this report (see Appendices 2 and 3) large numbers of wild rodent and flea species have been found infected in the various foci of the United States. Attempting a classification of the former, Eskey and Haas, in their classical study on "Plague in the western part of the United States" (1940), came to the conclusion that at least three groups of rodents constituted the great primary reservoirs of the infection - the ground squirrels (Citellus), which were widely involved in the coastal regions and in the northern part of the Intermountain Plateau: the wood-rats (Neotoma), which formed the plague reservoir in the sourthern deserts, and finally the prairie dogs (Cynomys), which harbored the infection in the plateau region of Arizona and New Mexico. It deserves, however, great attention that Kartman and his associates, confirming findings recorded by Meyer in 1943, came to the conclusion that

> "Historically, emphasis has shifted from the large colonial rodents such as ground squirrels to the small, inconspicuous native field voles and mice such as Microtus and Peromyscus."

It is of great importance to note that wild-rodent fleas have been found upon several occasions on the common rats and that isolated instances of rat plague conveyed by such fleas have been detected. Thus Meyer and Holdenried (1949), working on a Californian ranch where wild-rodent plague was present, found the infection also in R. rattus and R. norvegicus which were in part infested with ground-squirrel fleas. Observations in point were also recently made by Kartman and associates who were able experimentally to confirm the transition of wild-rodent fleas to rats with radioactively tagged Malaraeus telchinus. It is, however, reassuring to find that thus far the recent strictly sporadic human infections with P. pestis were invariably of the pattern of purely sylvatic plague, having been contracted away from the settlements in the wild-rodent foci. Thus the only distressing feature of the situation is that owing to the paucity of the human attacks quite often the occurrence of human plague is not recognized early enough to save the patients or a diagnosis is even arrived at only after the death of the victims. To alert the medical profession and the public in this respect is therefore an important task.

REFERENCE LIST

Meyer, K. F. et al. Sylvatic plague studies. J. inf. Dis. 73(1943)144-157.

Meyer, K. F. and Holdenried, R. Rodents and fleas in a plague epizootic in a rural area of California. Porto Rico J. of publ. hlth and trop. med. 24(1949)3:201-209.

Link, V. B. Plague. CDC Bull. 9(1950)8:1-14.

- Plague in North America Bol. Ofic. san. Panamer. 30(1951) 26.

- Plague. Amer. J. trop. Med. 31(1951)4:452-457.

- A history of plague in the United States of America. Publ. Hlth. Monogr. No. 26 (1955).

Kartman, L. et al. Ecological studies of wild rodent plague in the San Francisco bay area of California. Amer. J. trop. Med. and Hyg. 7(1958)1:112-114.

- New knowledge on the ecology of sylvatic plague. Annals N. Y. Acad. of Sci. 70, Art. 3(1958):668-671.

APPENDIX 1

HUMAN CASES OF PLAGUE BY RODENT SOURCE

CALIFORNIA 1900-1961

YEARS	TOTAL	SOURCE		
		RATS	WILD RODENTS	UNKNOWN
Total 1900-1959	409	342	56	10
1900	22			
1901	32	32		
1902	42	39		3
1903	20	17	3	
1904	10	10		
1907	180	178	1	1
1908	9	4	3	1
1909	4		3	1
1910	2		2	
1911	5		3	2
1913	2		2	
1914	1		1	
1915	1		1	
1919	14		14	
1920	1		1	
1921	2		2	
1922	2		2	
1923	1			1
1924	38	38		
1925	2	2		
1927	1		1	
1928	3		3	
1933	1		1	
1934	1		1	
1936	3		2	1
1937	1		1	
1941	2		2	
1942	1		1	
1943	1		1	
1944*				
1947	1		1	
1956	1		1	
1959	2		2	

Years in which no cases were reported are omitted

* 1 Lab. infection - San Francisco

APPENDIX 2

LIST OF WILD RODENTS AND LAGOMORPHA FOUND NATURALLY PLAGUE-INFECTED IN THE WESTERN UNITED STATES

FAMILY AND SUBFAMILY	SPECIES	STATE
RODENTS:		
Geomyidae (Gophers)	Thomomys bottae	California, ?Colorado
	Th. fossor	Colorado*
	Kangaroo rats:	
Heteromyidae Dipodomyinae	Dipodomys sp.	Texas
	Dipodomys ordi	Washington
	Perognathus parvus	Washington*
	Wood rats:	
Muridae Cricetinae	Neotoma albigula	Arizona, New Mexico
	N. cinerea occidentalis	California
	N. desertorum	Nevada, Utah
	N. fuscipes	California, Oregon*
	N. f. mohavensis	Nevada
	N. intermedia	California
	N. lepida	? Utah
	N. micropus	Texas
	Grasshopper mice:	
	Onychomys sp.	Texas*
	O. leucogaster	New Mexico* and other Western areas*
	O. torridus	New Mexico*
	Peromyscus boylii	Arizona*
	P. leucopus	New Mexico*
	P. maniculatus	New Mexico, California* Washington*
	P. truei gilberti	California
	P. truei truei	California, New Mexico
	P. truei nevadensis	Utah
	Reithrodontomys megalotis	California*, Kansas* New Mexico*
	Sigmodon hispidus	New Mexico*
	Voles:	
Muridae Microtinae	Lagurus crutatus	Washington*
	Microtus californicus	California
	M. montanus	Oregon, Washington*
	M. nanus	Washington*
	M. townsendi	Washington
	Squirrels:	
Sciuridae	Citellus armatus	Western part of U.S.A.
	C. beecheyi beecheyi	California
	C. b. douglasi	California, Oregon
	C. b. fisheri	California
	C. b. nudipes	California*
	C. beldingi beldingi	California
	C. b. oregonus	California, Nevada, Oregon

FAMILY AND SUBFAMILY	SPECIES	STATE
Sciuridae (cont)	Squirrels:	
	C. columbian/columbianus	Washington
	c.c. ruficaudus	Oregon
	C. idahoensis	Idaho*
	C. lateralis chrysodeirus	California
	C. lateralis	Wyoming*
	C. leucurus	Arizona*, California*
	C. mexicanus	New Mexico*
	C. richardsoni elegans	Wyoming
	C. r. nevadensis	Nevada
	C. r. richardsoni	Montana[a]
	C. spilosoma major	New Mexico*
	C. townsendi	Idaho*
	C. tridecemlineatus	New Mexico*, Texas*
	C. variegatus grammurus	Utah, Arizona*, Colorado* New Mexico*
	C. v. utah	Utah
	C. washingtoni loringi	Washington
	C. w. washingtoni	Washington
	Prairie dogs:	
	Cynomys sp.	Colorado*, Texas*
	Cynomys gunnisoni gunnisoni	New Mexico
	C. g. zuniensis	Arizona, New Mexico
	C. leucurus	Wyoming*
	C. ludovicianus	Colorado, Kansas, Montana, New Mexico, Texas, Wyoming
	C. parvidens	Utah[b]
	Flying squirrel:	
	Glaucomys sabrinus lascivus	California
	Marmots:	
	Marmota flaviventris subsp.	Colorado, Oregon New Mexico*[c]
	M. fl. avara	Oregon*
	M. fl. engelhardti	Montana, Utah, Wyoming
	M. fl. nosophora	Montana
	Chipmunks:	
	Tamias minimus	Washington*
	T. quadrivittatus frater	California, Nevada
	tamiasciurus douglasi	California
LAGOMORPHA:		
Leporidae	Rabbits:	
	Lepus californicus	California
	Sylvilagus audobonii	New Mexico
	S. bachmani	California*
	S. nuttalli	Washington

Remarks:- * The presence of natural plague infection was detected only in the fleas infesting these rodents

a Also found naturally plague-infected in Alberta and Saskatchewan, Canada

b A prairie dog, Cynomys mexicanus, was also found naturally plague infected in North Mexico

c Naturally plague-infected fleas were found on this subspecies in British Columbia, Canada

APPENDIX 3

LIST OF WILD-RODENT AND LAGOMORPHA FLEAS FOUND NATURALLY PLAGUE-INFECTED IN THE UNITED STATES

SPECIES	LOCALITY	USUAL HOSTS
Anomiopsyllus sp.	New Mexico	Neotoma
A. hiemalis	Texas	Neotoma
Atyphloceras sp.	Western part of USA	Lagurus, Peromyscus
A. multidentatus	Western part of USA	Peromyscus
Catallagia decipiens	Washington	Lagurus, Peromyscus
C. wymani	California	Microtus, Peromyscus
Diamanus montanus	Western part of USA	Citellus
Foxella ignota	Colorado	Thomomys
Hoplopsyllus anomalus	Western part of USA	Citellus
H. glacialis affinis	New Mexico	Sylvilagus
Hystrichopsylla linsdalei	California	Microtus
Malaraeus telchinus	Western part of USA	Microtus, Peromyscus
Megabothris clantoni	Washington	Lagurus, Peromyscus
Meringis shannoni	Washington	Lagurus and other wild rodents
Monopsyllus eumolpi	Western part of USA	Tamias
M. exilis	Texas	Onychomys
M. wagneri	Western part of USA	Lagurus, Peromyscus and other wild rodents
Opisocrostis hirsutus	Western part of USA	Cynomys
Opisodasis keeni nesiotus	California	Microtus, Peromyscus Reithrodontomys
Orchopeas leucopus	New Mexico	Peromyscus
O. neotomae	New Mexico	Neotoma
O. sexdentatus	Western part of USA	Neotoma, Peromyscus
Peromyscopsylla hesperomys adelpha	New Mexico	Peromyscus
Stenistomera macrodactyla	New Mexico	Peromyscus
Thrassis bacchi bacchi (= Thr. gladiolis)	Western part of USA	Citellus
Thr. bacchi johnsoni	Western part of USA	Lagurus, Peromyscus
Thr. fotus	Western part of USA	Onychomys
Thr. stanfordi	Western part of USA	Marmota

SECTION G

A LISTING OF NEEDS IN RESEARCH

1. PERU: Plague Ecological and Epidemiological Studies in the Huancabamba-Ayabaca focus.

1.1 WILD RODENTS AND LAGOMORPHA

1.1.1 Ecology studies

It is planned to study the ecology of the various species of rodents and lagomorpha involved in the plague manifestations, particularly in order to (a) delimit their habitat; (b) establish the possible existence of strongholds of the infection; and (c) to determine the aptitude of the animals to approach or to enter human habitations or settlements.

1.1.2 Occurrence of natural plague infection

It is proposed to make systematic wholesale studies on the occurrence of natural plague in the various species of rodents and lagomorpha, particularly in order to arrive at a distinction between reservoir species constantly harboring the infection and those getting periodically or occasionally involved.

1.1.3 Susceptibility to plague infection

As planned, batches of the species involved in the plague manifestations will be experimentally infected with P. pestis strains of standard virulence so as to determine their susceptibility for, or resistance against plague.

1.2 FLEAS OF THE WILD RODENTS AND LAGOMORPHA

1.2.1 Distribution of the various flea species

It is planned to make systematic studies so as to determine the occurrence and frequency of the various flea species concerned on (a) the wild rodents and lagomorpha; (b) the common rats, the house-mice and the domesticated guinea-pigs; and (c) in the various parts of the focus and at different seasons of the year.

1.2.2 Occurrence of natural plague in the various species

As projected, the occurrence of natural plague in the various fleas would be determined with the aid of pooling tests.

1.2.3 Vector capacity

It is proposed to study the vector capacity of the various flea species found naturally plague-infected, using whenever possible laboratory-bred strains.

1.3 COMMON RATS AND HOUSE-MICE

1.3.1 Systematic studies are planned to determine the presence or absence of the two species of the common rats and of the house-mice in both the settlements and in the rural areas of the focus.

1.3.2 Occurrence of natural plague

It is proposed to study the occurrence of natural plague in the common rats and the house-mice by (a) examining all animals found dead; (b) making guinea-pig tests with the pooled organs of trapped rats and mice.

1.3.3 Susceptibility to plague infection

Since it has been claimed that the Peruvian rats have become resistant to plague, it is planned to challenge batches of these animals, collected both in the settlements and in the rural areas of the focus, with P. pestis strains of standard virulence.

1.4 DOMESTICATED GUINEA-PIGS

1.4.1 Frequency studies

Observations would be made on the comparative frequency with which guinea-pigs are kept in (a) the settlements and (b) the rural houses of the various parts of the focus.

1.4.2 Occurrence of natural plague

It is proposed to keep a watch for the occurrence of natural plague in the domesticated guinea-pigs by (a) dissecting and examining all animals found dead; and (b) sacrificing and likewise examining those showing signs of illness.

The blood sera of all guinea-pigs under test will be kept in a lyophilized condition so as to make them available for further exhaustive studies.

1.4.3 Susceptibility to plague infection

As planned, it will be determined whether differences in susceptibility to experimental infection with various P. pestis strains exist (a) between the guinea-pigs kept respectively in recently plague-affected and in plague-free localities and (b) at different seasons of the year.

1.5 INTRADOMESTIC FLEA FAUNA

1.5.1 Occurrence of the various flea species

It is projected to make studies on the occurrence and frequency of the various flea species infesting (a) the common rats; (b) the house-mice and (c) the domesticated guinea-pigs as well as (d) on the fleas found free-living in the houses, paying special attention to the occurrence of (i) X. cheopis; (ii) wild rodent fleas and (iii) the various forms of P. irritans, the existence of which has been recently claimed.

1.5.2 Occurrence of natural plague

According to the plan, pooling tests would be made with batches of the various domestic fleas to determine the presence of plague in these parasites, particularly in localities invaded or threatened by plague.

1.5.3 Vector efficiency studies

It is proposed to make vector efficiency studies with the intradomestic flea species, particularly with the various forms of P. irritans met with.

1.5.4 Action of insecticides

Comparative studies are also contemplated of the action exerted by DDT and other insecticides on the domestic flea fauna.

1.6 EPIDEMIOLOGICAL OBSERVATIONS

1.6.1 Observations on manifest plague attacks

As planned, systematic studies would be made on all human manifestations of plague met with by the study group.

1.6.2 Subclinical forms of plague

It is proposed to study the occurrence of subclinical forms of plague with the aid of surveys in groups of the population exposed to the risk of infection.

1.6.3 Forecasting of plague manifestations

As set forth in the plague plan for Peru (see Section 9.5) it is planned to explore the possibility of forecasting the occurrence of human plague manifestations.

2. ECOLOGICAL AND EPIDEMIOLOGICAL STUDIES IN THE VENEZUELAN PLAGUE FOCUS

2.1 Studies analogous to those outlined above are also contemplated for the Venezuelan plague focus.

2.2 Pilot study on the control of wild rodent plague

Provided that strongholds of the infection are found in the Venezuelan plague focus, a pilot study is planned to explore the possibility of sufficiently thinning out the populations of the reservoir species in these localities so as to cut short the spread of plague. Consultations with wild-life experts would be indispensable to devise methods suitable for such a temporary reduction of the populations concerned which, as proved by experiences in the Soviet Union, is an effective method of plague control.

3. ECOLOGICAL AND EPIDEMIOLOGICAL STUDIES IN THE ECUADORIAN PLAGUE FOCI

In view of the involved plague situation in Ecuador it is proposed to postpone the framing of a program for studies in that country until experiences on the suitability of the plans contemplated for Peru and Venezuela have been gathered.

4. PLAGUE CONTROL

Since the aim of the proposed investigations is not to elicit facts of merely academic interest but to serve practical ends research would always be directed towards the ultimate goal of providing the knowledge and tools to permit the national plague prevention services to deal with imminent or actual manifestations of the disease in man. Attention would be paid in particular to studies on the possibilities of preventing human plague not only by the use of insecticides (see 1.5.4 above) but also with the aid of potent synergist vaccines in basic and booster doses and, whenever indicated by local emergencies, through administration of antibiotics or sulfonamides. The therepeutic use of these substances would likewise be studied as much as possible so as to arrive at a fully effective yet simple and economical scheme of treatment.

SECTION H

OUTLINE OF A RESEARCH PLAN FOR STUDIES ON THE ECOLOGY AND EPIDEMIOLOGY OF PLAGUE IN THE HUANCABAMBA-AYABACA FOCUS, PERU

1. General organization of the work

In order to cope with the involved problems to be investigated, the proposed study will be made on four levels:

(a) In the field;
(b) In a base laboratory at Lima, Peru;
(c) In one of the plague laboratories of the U.S.A. where the plague strains isolated in the field or at the base laboratory will be studied and tests with the sera of plague patients, contacts and rodents requiring special techniques will be made;
(d) In three reference laboratories, namely the CDC Field Station at San Francisco, where the vector efficiency of the various species of fleas of the focus playing a role in the ecology of plague will be determined; the U.S. Natural History Museum in Washington, D.C., where final identifications of the different rodents and lagomorpha met with in the course of the study will be made; and the branch of the British Museum in Tring (England), where the identifications of the fleas collected in the course of the work by the field and base laboratory staffs will be confirmed.

The division of duties between the field staff and the workers at the base laboratory will be made evident when discussing the various parts of the research program.

2. Personnel required

The personnel necessary for the proposed work is enumerated in the following rooster:

International staff	Peruvian staff	Collaborating laboratories
1 Ecologist (team leader)*	1 Epidemiologist	It is expected that the collaborating laboratories will delegate members of their own staff for the required work, but funds must be available to enable them to come for necessary consultations to Washington
1 Bacteriologist*	1 Bacteriologist	
1 Mammologist*	2 Clinicians (part-time)	
1 Entomologist (flea expert)*	1 Secretary	
2 Field Supervisors*	4 Qualified Field Workers	
1 Part-time Consultant*	6 Trappers*	
	2 Drivers*	
	Laborers	

* To be paid out of the research grant

In order to assure adequate assistance for this study, key personnel of the Peruvian staff will be given some preliminary training abroad.

3. Length of the study period

In view of the large scope of the research program and bearing in mind the seasonal changes of the ecological situation, the work of the team must be planned for a period of not less than two years, the first year to be used for obtaining an overall picture of the situation, the second for focussing attention upon the solutions of problems found to be of particular importance.

4. Investigations on wild rodents and lagomorpha

In order (i) to investigate which species of wild rodents and lagomorpha suffer from natural plague; (ii) to establish which of these species is or are the permanent reservoir of the infection and (iii) to ascertain the trend of the infection in the species involved the following investigations are necessary:

4. 1) To examine all animals found dead. These after they have been preliminarily identified and after their fleas and other ectoparasites have been collected and put into individual vials, will be dissected by the field staff who (a) will note and record all gross signs suggestive of plague (b) will make impression films from the buboes (if present) and the spleen, liver and lungs as well as smears from the heart blood and (c) put the bubo, heart and spleen (or a piece thereof) as well as pieces of the liver and lungs into a jar containing 2% saline solution or Broquet's fluid. In the case of decomposed or mutilated carcasses, one or a few marrow bones will be put into the preserving liquid. After the impression films and smears have become air-dry, the properly labelled slides will be put into a jar containing methylated alcohol. If the dissected animals are small, their carcasses will be put into a jar filled with methylated alcohol or 10% formol. In the case of larger animals their skulls and skins will be forwarded to the base laboratory, together with the above-mentioned material. In the base laboratory the identity of the dissected animals will be confirmed or determined and all necessary bacteriological and serological tests will be made to detect the presence of plague in the dissected animals. All P. pestis strains isolated will be kept in a lyophilized state for further study in the U.S.

4. 2) In order (i) to watch for the occurrence of incipient or latent infections in the wild rodents and lagomorpha and (ii) to obtain specimens for a determination of the susceptibility of the various species to infection with P. pestis, adequate numbers of all species present in the study areas will be trapped or obtained by other means.

4. 3) The major part of the trapped animals - if possible after a period of observation in the live state - will be sacrificed and material for tests for the presence of plague infection in the base laboratory will be obtained in the manner described in paragraph 4. 1). Examining this material in the base laboratory, ample use will be made of animal experiments with the pooled organs of lots of animals belonging to one and the same species.
The material from animals obtained by killing methods will be handled in an identical manner.

4. 4) Lots of not less than 6 animals of the various trapped species will be disinsected, serologically tested and - if possible after a period of observation - will be challenged with adequate doses of a plague strain of standard virulence. It would be desirable to perform these tests in the base laboratory by forwarding to it animals trapped in localities free from epizootics at the time in flea-proof cages.

4. 5) While it is impossible to predict the number of animals found dead, it will be attempted to examine a total of not less than 100 rodents and lagomorpha per week or 5000-6000 animals per year.

4. 6) In addition to the above-described work ecological studies will be made to ascertain the distribution of the different species and the limits of their habitat and

4. 7) A constant watch will be kept for fluctuations in the population density of the various species with aid of enquiries among the population and the plague prevention staff, periodic inspections and systematic trapping operations in representative plots of standard size, repeated in the various localities at regular intervals.

4. 8) The observations on the sporadic, periodic or constant occurrence of positive findings in the various species in combination with the ecological observations and the results of the resistance studies will be used as a means to assess the importance of the different rodents and lagomorpha for the permanent harborage of the infection.

4. 9) By correlating the results of laboratory observations with those on the population density of the essential species the trend of plague in the focus will be gauged.

5. Investigations on the fleas of the wild rodents and lagomorpha

In order to ascertain the comparative importance of the various flea species of the wild rodents and lagomorpha in the Huancabamba-Ayabaca focus for the transmission of plague the following investigations will be made:

5.1) Flea collections will be made from (i) all rodents and lagomorpha found dead as well as from those trapped or collected by other means; (ii) rodent nests or burrows, and (iii) other sites suitable for the harborage of fleas.

5.2) The collected fleas will be forwarded to the base laboratory, using separate, adequately-labelled and well-closed vials for the flea lots collected from the individual rodents and lagomorpha, nests, etc.
Should it be impossible to forward the fleas alive, they will be put in a preserving fluid like saline solution so as to permit their examination for the presence of P. pestis.

5.3) In the base laboratory the fleas will be subjected to preliminary identification tests and divided into lots consisting of only one species. Representative specimens, preserved in alcohol or glycerol alcohol, will be sent to Tring for final identification.

5.4) Pools of fleas belonging to one and the same species will be used for the experimental infection of guinea-pigs so as to detect the presence or absence of P. pestis in the lots under test.

5.5) It will be attempted to handle about 200 fleas from wild rodents and lagomorpha per week or 10,000-12,000 per year.

5.6) As far as possible the species of fleas in which the occurrence of natural plague has been detected will be bred in the base laboratory and lots of the bred fleas will be forwarded to the CDC Field Station in San Francisco for vector studies.
In the case of species which cannot be bred in the laboratory, lots of the fleas in question collected in localities free from epizootics at the time will be sent to San Francisco.

5.7) Detailed accounts of the occurrence of the various flea species found on the different rodents and lagomorpha and in other sites will be kept so as to assess (i) the occurrence of the various flea species on the different species of rodents and lagomorpha, (ii) the location and the limits of the habitat of the various fleas, and (iii) the occurrence of seasonal fluctuations of their frequency.

5.8) The results of the examinations of the fleas for the presence of P. pestis and of the vector capacity studies correlated with the ecological observations (5.7) will be used to determine the comparative importance of the species in question for the transmission of plague.

5.9) In order to ascertain the epidemiological importance of the fleas thus incriminated, their ability to attack the common rats, the domesticated guinea-pigs and man will be ascertained.

6. Investigations on the common rats and domestic mice

Since it has been maintained so far that the common rats (R. rattus) (a) are not uniformly distributed in the Huancabamba-Ayabaca focus, being rare in the rural areas and (b) because being resistant to infection with P. pestis, play no role in the transition of the infection from the wild rodents and lagomorpha to man, the following investigations will be made:

6. 1) The comparative frequency of these animals throughout the focus will be studied through uniformly conducted trapping operations in and round the houses.

6. 2) Batches of at least 20 live specimens trapped in the various towns and rural areas of the focus will be used for uniformly conducted susceptibility tests with P. pestis strains of standard virulence.

6. 3) In localities where the occurrence of plague among the wild rodents and lagomorpha or in the human population has been recorded (a) a careful search will be instituted for rats and domestic mice found dead in or round the houses and any carcass found will be examined with the aid of the methods described in Section 4. 1) of this plan; (b) ample trappings will be made and the trapped rats and mice will be examined for the presence of incipient or latent infection, laying particular stress upon the performance of tests with their pooled organs. As will be stated below, the fleas collected from the rats and house-mice will also be used for pooling tests.

6. 4) It will be attempted to examine an average of 60-80 common rats and house-mice per week or about 3,000-4,000 per year.

7. Investigations on the domesticated guinea-pigs

7. 1) Whenever the presence of plague in wild rodents and lagomorpha is detected, a careful watch will be instituted to detect a transition of the infection to the domesticated guinea-pigs by (a) dissecting and examining any of these animals found dead and (b) sacrificing and likewise examining those showing signs of illness.

7. 2) Comparative studies will be made to establish whether differences in the susceptibility to experimental infection with P. pestis exist between the guinea-pigs kept in recently plague-affected settlements or houses (particularly in places where these animals themselves have been involved in the manifestations of the disease) and in localities recently free from plague. Incidental to these investigations it will be studied whether seasonal changes of the susceptibility to plague exist in the domesticated guinea-pigs.

8. Investigations on the intradomestic flea fauna

8. 1) A focus-wide study will be made of the fleas infesting the common rats, particularly in order (i) to delimit the areas in which X. cheopis infests these rodents; (ii) to establish what species replace this vector in localities unsuitable for its existence and (iii) to note the infestation of the common rats with fleas not specific to them, particularly with wild-rodent fleas and P. irritans.

8. 2) In the course of the just described work analogous observations will be made on the flea-fauna of the domesticated guinea-pigs and on the fleas infesting the house-mice. Particular attention will be paid to (i) the occurrence of wild-rodent fleas and P. irritans on these rodents; and (ii) the degree to which an interchange of fleas specific for the common rats, respectively for the guinea-pigs takes place.

8. 3) Making the above enumerated investigations, attention will be paid also to the occurrence and comparative frequency of the different forms of P. irritans on the rodents as well as in the houses in general.

8. 4) Using separate adequately-labelled vials for the lots of fleas from the individual rodents or locations, the flea material will be forwarded to the base laboratory for (i) identification and recording of the species met with; (ii) pooling tests to detect the presence of plague-infected fleas and (iii) breeding of the flea species, the vector capacity of which it is indicated to study.
In the case of species which cannot be bred in the laboratory (presumably in that of the various forms of P. irritans) lots of fleas collected in locations free from plague will be forwarded to the San Francisco CDC station for vector efficiency tests.

8. 5) Comparative studies will also be made of the action exerted by DDT and other insecticides on the domestic flea fauna.

9. Epidemiological observations

9. 1) A close study will be made of all human plague manifestations met with by the research team, particularly in order to establish which rodents and which fleas were involved in the causation of the human infections. Houses in which several plague attacks have occurred will be given special attention in this respect.

9. 2) The study group will stand ready to assist the Plague Prevention Service in obtaining in each instance of human plague a confirmation of the diagnosis through laboratory tests. The plague

strains isolated in the course of this work will be kept in lyophilized form for further study in a reference laboratory.

9. 3) In order to obtain complete and uniform records an adequately prepared record sheet will be used for each patient. The help of the Plague Prevention Service will be enlisted to fill out such questionnaires also for patients seen by them in the recent past, and the future use of these record sheets will be recommended.

9. 4) Efforts will be made to detect the occurrence of subclinical forms of plague through physical and serological surveys in groups of the population which have been or are under the risk of infection.

9. 5) As discussed at the conclusion of the report on Peru, attention will be given to forecasting the incidence of human plague through a correlation of all available data on the fluctuations of the population density of the rodent hosts of the infection and on the occurrence and frequency of manifestations of the disease in the herds of these animals.

APPENDIX

PRELIMINARY STEPS TO BE TAKEN FOR THE WORK IN THE HUANCABAMBA-AYABACA

In order to facilitate discussions and arrangements with the Peruvian officials, to make any necessary amendments to details in the project plans, and to prepare staff for the research work, the following activities are deemed necessary:

1. Preliminary studies and arrangements in Peru. A staff member of PASB, the Team Leader and possibly the part-time consultant (as short-term consultant of PASB) should go to Peru at the earliest convenient date to undertake the preliminary studies and arrangements, including:

1. 1) Following visits to the project site areas the research plan will be discussed with appropriate technical persons in Peru and amended as indicated.

1. 2) Personnel, facilities and services to be provided by the government of Peru will be discussed in detail and tentative arrangements made.

1. 3) A form of official agreement between the government and the Organization will be drafted with the assistance of appropriate Peruvian officials.

1. 4) The latest available data on plague in man and animals in Peru will be obtained.

2. Following the visit to Peru the plan of operation for the research project will receive final revision and an application submitted to NIH, USPHS for a research grant.

3. In the period during which the application is being processed and considered discussion of tentative arrangements will be continued with the collaborating laboratories and agencies.

4. When, and if the research grant is made pre-field activity will begin which will include:

4. 1) The Mammalogist will spend a period in the Natural History Museum, Washington D.C., where he will:

4. 1) 1. Become familiar with the specimens in the collection of rodents from Peru, and

4. 1) 2. Determine the technique desired by the Museum for the

preparation and shipment of specimens which will be sent for identification.

4. 2) Obtain from the British Museum, Tring, England, descriptions and mounted specimens of fleas which have been obtained from the focus area in Peru, so that the Entomologist can acquaint himself with this material.

SECTION I

OUTLINE FOR A RESEARCH PLAN FOR PLAGUE INVESTIGATIONS IN VENEZUELA

In accordance with the recommendations contained in the Section C on Venezuela, a plan for the investigation of the ecology and epidemiology of plague in the presently affected areas of Venezuela may be itemized as follows:

1. Investigation on wild rodents and lagomorpha, comprising
   1.1) Examination of all animals found dead for the presence of plague.

   1.2) Collection of large numbers of apparently healthy animals by trapping or (as far as necessary) by other means so as to obtain material for
      1.2)1. Pooling tests with the organs of sacrificed trapped animals or animals obtained by killing methods so as to detect the presence of plague infection.

      1.2)2. Determination of the susceptibility of the various species to experimental infection with P. pestis strains of standard virulence.

   1.3) Ecological studies to ascertain the distribution of the different species and the limits of their habitat.

   1.4) Observations on fluctuations of the population density of the various species.

2. Investigations on the fleas of the wild rodents and lagomorpha by

   2.1) Making flea collections from (i) all rodents and lagomorpha found dead, trapped or collected by other means; (ii) rodent nests or burrows; and (iii) other flea harborages.

   2.2) Using pooled lots of fleas belonging to one and the same species of the various fleas met with for the experimental infection of guinea-pigs so as to detect the presence of infected fleas. Cultures will also be made from the fleas to detect the presence of avirulent strains.

   2.3) Breeding fleas of the species found to be plague-infected in the laboratory and using the bred fleas for vector efficiency studies.

3. Investigations on the common rats and mice, including

   3.1) Studies on the occurrence and comparative frequency of R. norvegicus and R. rattus together with observations on differences in the ecology of these two species.

   3.2) Tests to detect the presence of plague in these species and the house-mice in localities where plague has become manifest (examination of all rats and mice found dead and of trapped or killed animals).

   3.3) Uniformly conducted susceptibility tests with P. pestis of standard virulence to determine to what extent the rats trapped in the various localities of the focus have become resistant to plague.

4. Investigations of the domesticated guinea-pigs, comprising

   4.1) Observations on the frequency with which these animals are kept in the various localities of the focus.

   4.2) Tests to detect the presence of plague in these animals in localities where plague has become manifest.

   4.3) Comparative studies to establish whether differences in the susceptibility to experimental infection with P. pestis exist between guinea-pigs kept in recently plague-affected settlements or houses and in localities free from plague.

5. Investigations on the intradomestic flea fauna, consisting of

   5.1) Studies to determine the species of fleas habitually or accidentally infesting the common rats, the house-mice, the domesticated guinea-pigs and also the marsupialia.

   5.2) Pooling tests with the various species of the intradomestic fleas in localities where the occurrence of plague has been detected.

   5.3) Vector efficiency studies with the species of the intradomestic fleas which seem to be of importance in the conveyance of plague.

   5.4) Comparative studies of the action of DDT and other insecticides on the intradomestic flea fauna.

6. Epidemiological investigations, comprising

   6.1) Thorough clinical studies and laboratory examinations of any human plague case met with. The efficacy of antibiotics and sulfonamides for the prevention and treatment of plague will also be studied.

   6.2) Efforts to detect the occurrence of subclinical forms of plague through physical and serological surveys in groups of the population which have been or are under the risk of infection.

   6.3) Attention to the possibility of forecasting the incidence of human plague through a correlation of the data on the fluctuations of the population density of the rodent hosts of the infection and the occurrence and frequency of manifestations of the disease in the herds of these animals.

7. Evaluation and utilization of the results of the investigations.

   The results of the above-outlined investigations will be used

   7.1) To determine the present limits of the plague-affected area.

   7.2) To establish whether within the affected area strongholds of the infection exist.

8. Basing upon these data it will be decided whether a pilot study to assess the possibilities of a campaign against the wild rodents would be justified.