Bull. Org. mond Santé
Bull. Wld Hlth Org. } 1965, 33, 809-815

Results of a Preliminary Serological Survey of Small Mammal Populations for Plague on the Island of Hawaii*

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Since 1910 the District of Hamakua, Island of Hawaii, has been considered an endemic plague area. To obtain indirect evidence of plague infection in rodents and in the mongoose, serological surveys of the small mammal populations were undertaken. The passive micro-haemagglutination test demonstrated the presence of positive reactors (titres of 1 : 16 and higher) in the sera of 2641 rodents and 385 mongooses tested. Positive percentages were : Rattus exulans, *1.5 % ;* R. norvegicus, *1.9 % ;* R. rattus, *0.6 % ;* Mus musculus, *1.1 % ; and* Herpestes auropunctatus, *12.5 %. This study has proved the continued presence of a permanent reservoir of plague in indigenous rodent and mongoose populations after a period of 65 years since the introduction of plague into Hawaii and has demonstrated that the mongoose is an excellent indicator of plague infection.*

Plague in the Hawaiian Islands was first encountered in 1899 and is at present endemic in several districts of the Island of Hawaii. Of the 410 human cases reported in the Islands since then, 112 cases with 109 deaths occurred in the Hamakua District, where plague has been endemic since 1910; the last case was reported in 1949. The enzootic persistence of the infection has been manifested in the rodent and flea populations, from which 1161 isolations of *Pasteurella pestis* have been made by public health authorities during the past fifty years (*Rattus exulans*, 453; *R. rattus*, 292; *R. norvegicus*, 195; *Mus musculus*, 96 and 125 flea pools). Although the infection has previously remained quiescent for long periods, the current period beginning in May 1957 is the longest on record in which infected fleas and rodents have not been detected (Table 1).

In an attempt to determine the factors underlying the past persistence of plague and to explain its current quiescence, the Rodent Control Branch, Department of Health of the then Territory of Hawaii, initiated an intensive ecological study of plague in 1958 in the Hamakua District. As no isolations of *P. pestis* had been made during the past eight years, susceptibility tests of rodents to plague infection were undertaken as one of two avenues of study to provide indirect evidence of plague infection in nature. The second approach was by a serological survey of the small mammal populations of the Island of Hawaii for the presence of antibodies against Fraction I antigen of *P. pestis*. The late Dr Stuart F. Quan of the Communicable Disease Center, San Francisco Field Station, in a co-operative venture with the Department of Health, State of Hawaii, demonstrated that *R. norvegicus* and *R. exulans* from the Island of Hawaii were significantly more resistant to experimental plague infection than their counterparts from plague-free Oahu. No such difference was observed in *R. rattus*. As resistance could not be correlated with sex or age of the animals or season of collection, the observed differences were apparently due to geographical factors associated with the presence or absence of enzootic plague (Quan et al., 1965).

* This investigation was supported, in part, by Grant No. AI-02886-06 TMP from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, US Department of Health, Education, and Welfare, and by the Department of Health, State of Hawaii. The study was undertaken in co-operation with the George Williams Hooper Foundation, University of California Medical Center, San Francisco, Calif., and was supported, in part, by Contract No. DA-49-193-MD-2121 from the Commission on Immunization, US Armed Forces Epidemiological Board.

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TABLE 1

HISTORICAL SUMMARY OF PLAGUE CASES AND DEATHS, AND OF RODENT AND FLEA ISOLATIONS IN THE HAWAIIAN ISLANDS

Locality [a]	Date when plague first detected	Total cases	Deaths	Date of last human case	No. of rodent and flea isolations	Date of last rodent and flea isolations
Oahu	Dec. 1899	228	204	1910	?	1907
Maui	Jan. 1900	16	15	1938	64	1951
Kauai	Nov. 1901	11	11	1906(?)	?	?
Hawaii						
Hilo	Feb. 1900	43	24	1910	15	1918
Hamakua	March 1910	112	109	1949	1 161	1957
Total		410	363		1 240	

[a] The districts of Makawao, Maui and Hamakua, Hawaii, are at present considered endemic plague foci.

METHODS

Rodents and mongooses were live-trapped in each of the six districts of the Island of Hawaii at elevations ranging approximately from 15 to 2300 feet (about 4 m to 700 m) above sea level, killed with cyanide fumes and, except for specimens of *Mus musculus*, bled directly from the heart. Clear serum samples were stored in a deep-freeze until used. Heart-blood specimens from *Mus musculus* and from small, difficult-to-bleed individuals of the *Rattus* species were collected by absorption with 17-mm absorbent-paper discs (Trainer et al., 1963). The elution procedure yielded serum diluted 1:4, requiring the test to be started at a dilution of 1:16.

The techniques used were those recommended by Baltazard et al. (1956) and Meyer et al. (1963) for the complement-fixation and passive haemagglutination tests, adapted to the micro-techniques recommended by Sever (1962).

To determine the specificity of the haemagglutinin reaction, a series of 12 mongooses trapped near rubbish dumps of Kailua, Kona, and kept in captivity at the laboratory at Honokaa, were inocu-

TABLE 2

RESULTS OF SEROLOGICAL SURVEY OF SMALL MAMMALS, ISLAND OF HAWAII

Species	Total	Reactors [a]	Positive [b]	Titres			
				1:16	1:32	1:64	1:128
Rattus exulans	964	78 (8.1 %)	14 (1.5 %)	9	3	1	1
R. rattus	783	30 (3.8 %)	5 (0.6 %)	3	1	1	
R. norvegicus	264	18 (6.8 %)	5 (1.9 %)	1	2	1	1
Mus musculus	630	— [c]	7 (1.1 %)	1	5	1	
Herpestes auropunctatus	385	128 (33.2 %)	48 (12.5 %)	26	11	11	
Total	3 026	254	79				

[a] Titres of 1:4 and above.

[b] Titres of 1:16 and above.

[c] Disc samples *Mus musculus* (beginning dilutions 1:16) not included under " Reactors ".

TABLE 3
POSITIVE REACTORS BY SMALL MAMMAL SPECIES AND MONTH, ISLAND OF HAWAII

Species	1964 [a]								1965					Total
	April	May	June	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	
R. exulans		2	1	1	1			1	1	4	1	2		14
R. rattus					1					1	1	2		5
R. norvegicus	1					3					1			5
M. musculus					1	1		5						7
H. auropunctatus	1		1		1	3	3		8	26		1	4	48
Total	2	2	2	1	4	7	3	6	9	31	3	5	4	79

[a] No samples were taken in July 1964.

lated with a standard plague vaccine (virulent formol-killed and $Al(OH)_2$-coated *P. pestis* 195/P) used for studies on the immunization of man against plague. Blood specimens were tested for antibodies at intervals of 14, 28, 72 and 96 days after inoculation.

Sera were examined and all tests were analysed at the George Williams Hooper Foundation, University of California Medical Center, San Francisco, California.

RESULTS AND DISCUSSION

The results were critically analysed. Rodent sera yielding titres of 1:4 and over were considered " reactors ", while those of 1:16 and over were definitely " positive ". Table 2 shows that of the 3026 serum and disc samples tested, 254 (8.9%) were reactors, of which 79 (2.6%) were positive. Of the 630 samples from *Mus musculus* 7 (1.1%) with titres of 1:16 to 1:64 were positive. Most unexpected were the test results of sera from the mongoose, *Herpestes auropunctatus*, with 33.2% reactors and 12.5% positive, their titres ranging from 1:16 to 1:64.

Chronologically, positive reactions have been obtained from rodent and mongoose sera in each month of the year in which samples were collected and tested. The findings shown in Table 3, however, reveal the interesting fact that the majority fell in the period from October to March with a definite peak in February. In the past this was the season when most of the *P. pestis* isolations and the highest peaks in the flea populations occurred in Hamakua.

Geographically, serum reactions have been found in small mammals from widely scattered areas on the 4039 square miles (about 10 450 km²) of the Island of Hawaii. Since the collections for the most part have been small, insufficient evidence has been amassed to conclude that any given area has a greater concentration of reactors than any other. In several localities on the Island, however, there appear at present to be concentrations of serum reactors among the small mammal populations (see Table 4 and the map). Serological techniques, if used in the future as a method of plague surveillance in the State of Hawaii, should produce data which would delineate definite areas of concentration.

In response to plague vaccine, the mongoose develops specific Fraction I antibodies in the peripheral blood in titres similar to those of guinea-pigs. A relationship between the antigenic mass and the serum titres is indicated. Individual mongooses inoculated with a small dose show a decline of antibodies within 28-96 days while the antibodies persist in animals treated with larger dosages. Their serum reactions indicate that some of the animals were previously infected in nature, as they gave " booster " responses—rapid rise and corresponding speedy decline in titre (Table 5). The general trend of serum antibodies to reach lower titres by the end of the third month after inoculation applies to mongooses not previously infected and fully protected against infection during the experiment. The serological behaviour pattern of mongooses obviously recovered from natural infection (No. 11-15) before being inoculated with killed plague bacilli is noteworthy. Restimulation with a fairly large anti-

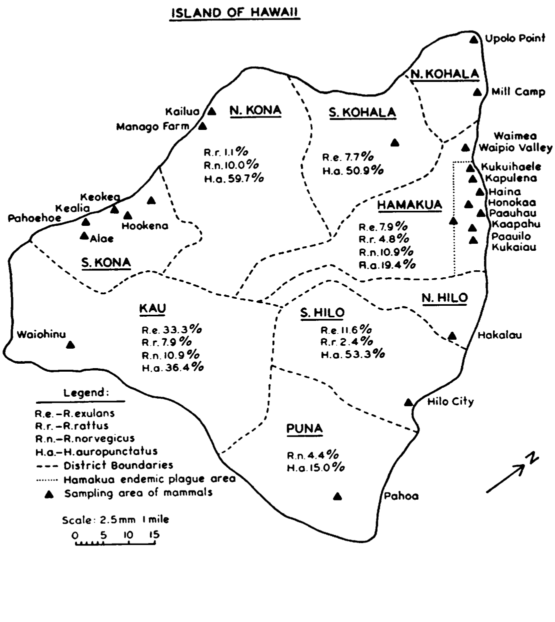
TABLE 4

RESULTS OF SEROLOGICAL SURVEY OF SMALL MAMMALS, ISLAND OF HAWAII, BY DISTRICT

Species	Sera [a]	District						Total
		Hamakua	Hilo	Puna	Kau	Kona	Kohala	
R. exulans	T	887	43	9	6	6	13	964
	R	70	5		2		1	78
	P	13	1					14
R. rattus	T	421	41	22	89	188	22	783
	R	20	1		7	2		30
	P	4				1		5
R. norvegicus	T	64	42	91	55	10	2	264
	R	7		4	6	1		18
	P	2		2	1			5
Mus musculus	T	569	6	8	8	1	38	630
	R [b]	X	X	X	X	X	X	X
	P	7						7
Herpestes auropunctatus	T	211	15	20	11	77	51	385
	R	41	8	3	4	46	26	128
	P	16	4	1	1	18	8	48
Total	T	2 152	147	150	169	282	126	3 026
	R	138	14	7	19	49	27	254
	P	42	5	3	2	19	8	79

[a] T = Total. R = Reactors. P = Positive.
[b] X = Disc samples (starting dilution 1 : 16); not included in " Reactors ".

DISTRIBUTION OF PLAGUE SERUM REACTORS AMONG SMALL MAMMAL POPULATIONS



genic mass of 500 million *P. pestis* raises and maintains the titre. Under the ecological conditions prevailing on the Island of Hawaii, reinfections are likely to occur during the months of November to April, when serologically recognized plague is prevalent in the rat population. Consequently a high titre demonstrated in a single test on a mongoose may be the consequence of repeated infection and antigenic stimulation. Observations made in connexion with experimental infection of *R. norvegicus* trapped on the Island support this conclusion.

Although the specificity of the serological reaction of mongoose sera was indirectly proved, it was equally important to search for additional proof that these carnivores are susceptible to plague. The mongoose was introduced into Hawaii in 1883 for rat control work and proved susceptible to plague by inoculation experiments in 1910 (Mott-Smith, 1910). Spontaneous plague infection in a trapped mongoose was reported in 1912 (by McCoy, referred to by Gross & Bonnet, 1951). How this species became infected is unknown, and the question has never been investigated. Indian species of the genus were

TABLE 5

HAEMAGGLUTINATION ANTIBODY RESPONSE TO INJECTION OF $Al(OH)_3$-COATED PLAGUE VACCINE IN FOUR REACTOR AND EIGHT NON-REACTOR MONGOOSES

Mongoose No.	Vaccine [a] amount	Haemagglutination titres				
		Pre-injection	Post-injection (days)			
			14	28	72	96
			Negative reactors			
3	1.0 ml	0	1 : 128	1 : 512	Died	—
5	1.0 ml	0	1 : 64	1 : 512	1 : 64	1 : 128
8	0.5 ml	0	1 : 16	1 : 512	1 : 64	1 : 128
16	0.5 ml	0	1 : 128	1 : 32	1 : 8	1 : 4
17	0.1 ml	0	1 : 512	1 : 128	1 : 64	1 : 128
18	0.1 ml	0	1 : 128	1 : 16	0	1 : 4
19	0.01 ml	0	1 : 64	1 : 4	1 : 8	1 : 16
20	0.01 ml	0	1 : 4	NT [b]	0	0
			Pre-vaccinal reactors			
11	0.2 ml	1 : 8	1 : 128	1 : 128	1 : 16	1 : 64
12	0.2 ml	1 : 8	1 : 256	1 : 256	1 : 64	1 : 128
14	0.2 ml	1 : 32	1 : 512	1 : 32	1 : 4	1 : 4
15	0.2 ml	1 : 4	1 : 32	1 : 128	1 : 16	1 : 8
			Controls			
7	— [c]	0	1 : 16	Died	—	—
13	— [c]	0	0	0	1 : 8	1 : 8

[a] 2.3×10^9 organisms/ml.
[b] Not tested.
[c] 1 ml of saline solution.

reported by the German and Austrian Plague Commissions in 1899 and 1910 (see Simpson, 1905) to be highly susceptible. Well-documented experiments and observations on the South African yellow mongoose (*Cynictis penicillata*) by Thornton (1933) indicate that this carnivore is rather refractory to *P. pestis* and that cases of spontaneous plague are rare. On the other hand, the suricate (same subfamily) was found more sensitive—" dying readily either from toxaemia alone or with actual infection " (Pirie, 1927). Equally important is the observation (Mitchell, 1924) that the mongoose may pick up large numbers of infected fleas and scatter them as it moves about. According to Fourie (1938), the yellow mongoose normally feeds on termites and other insects, but when rodents are lying about dead, either in warrens or in the open, they are quickly snapped up by the mongoose and their excrement transferred from insect chitin to rodent fur. " This change is one of the most useful field indicators of mortality in wild rodents and is often invoked to support deductions made from other signs of mortality."

Recent studies [1] of the content of the stomach and intestines, as well as of the stools, of the mongoose in Hawaii show that this animal is a rather omnivorous feeder. Mongooses have been found to consume large centipedes, various insects (dung beetles), coconut particles from trap bait, fish heads or flesh, various kinds of fruits and seeds, both wild and domestic birds, and rodents. The faecal content of the mongoose varies from one area to another.

[1] H. T. Kami, Member of the Plague Research Unit, Department of Health, State of Hawaii—unpublished studies, 1965.

Within the Hamakua District it was composed of insect remains and seeds of fruits. In the Waipio Valley, which delineates the north-western boundary of the known plague endemic area of Hamakua on the rim of the valley where *P. pestis* isolations were last obtained in 1957, the intestinal and faecal content consisted chiefly of rodent remains. Provided that the observations of Fourie on *Cynictis penicillata* can also be applied to *Herpestes auropunctatus*, it may be concluded that the stool content reflects mortality in rodents. The serological survey in this area lends support to this inductive interpretation of the faecal findings. Of 31 mongoose sera tested, 9 (29%) were reactors and 4 (12.9%) were positive with titres up to 1:64. In comparison with this, out of 144 *R. exulans* tested 8 (5.5%) were reactors and 2 (2.1%) were positive; the reactor percentage is slightly lower than for the Hamakua District. These observations suggest that in the course of the quiescent epizootic, recovery from plague infection in *R. exulans* may be less frequent in the Waipio Valley than in the Hilo District (see the map).

Similar findings and four " unsuspected " plague areas were revealed by serological survey in other districts on the Island of Hawaii outside the Hamakua District. In the Kona District, of 77 mongoose sera tested, 46 were reactors (59.7%) with 18 positive (23.4%); of 188 sera from species of *Rattus*, two were reactors (1.1%) but only one was positive. In the District of Kohala in the north-west corner of the Island, of 51 mongoose sera tested, 26 (51.1%) were reactors with 8 (15.7%) positive, but only one reactor was found among the rodent species tested. In the Districts of Kau and Puna, a trend in the same general direction was evident, but the number of animals captured was too small to provide a basis for comparison.

A comparison of these preliminary serological findings on mongoose and rodent sera shows with considerable certainty that the mongoose may be an excellent indicator of plague infection. In any given area on the Island of Hawaii this animal serves within its home range as a " selective host medium " for *P. pestis* garnered from rodent hosts. It is unknown whether classical vector transmission or infection through ingestion of rats sick or dead from plague plays a role in the infection of the mongoose. Perhaps its intestinal tract may supply specimens needed to achieve the isolation of *P. pestis* if pools of vectors or tissues continue to prove negative.

RÉSUMÉ

Depuis la première apparition de la peste dans les îles Hawaii en 1899, il y eut 410 cas humains, dont 112 avec 109 décès dans le district d'Hamakua. La persistance d'une enzootie s'est manifestée depuis lors chez les rongeurs et les puces, mais aucun ne fut trouvé infecté depuis mai 1957. Les auteurs ont étudié la sensibilité des rongeurs à l'infection et mené une enquête sérologique chez les populations de petits mammifères pour rechercher la présence d'anticorps contre la fraction antigénique I de *Pasteurella pestis*.

Des rongeurs et des mangoustes ont été capturés, tués par inhalation de cyanure et saignés. Douze mangoustes conservées au laboratoire ont été inoculées avec un vaccin antipesteux standard et les anticorps ont été titrés 14, 28, 72 et 96 jours après l'inoculation. Des 3026 sérums d'animaux sauvages examinés, 254 (8,9%) ont réagi, avec un titre de 1/4 et plus; 79 (2,6%) ont été nettement positifs, avec un titre de 1/16 et plus, en utilisant les réactions de fixation du complément et d'hémagglutination passive. Sept (1,1%) des 630 sérums de *Mus musculus* ont été positifs avec des titres de 1/16 à 1/64. Les résultats ont été inattendus chez la mangouste, *Herpestes auropunctatus*, avec 33,2% de sérums réagissants et 12,5% de sérums positifs à des titres compris entre 1/16 et 1/64. Les réactions positives ont été trouvées pendant toute l'année, la majorité cependant entre octobre et mars, avec un pic en février. C'est à cette époque que, dans le passé, l'on isolait le plus fréquemment *P. pestis* et que la population pulicidienne était la plus nombreuse. L'immunisation expérimentale des mangoustes et les observations sérologiques font conclure qu'un titre élevé d'anticorps chez la mangouste peut être la conséquence d'infections et de stimulations antigéniques répétées; les anticorps vaccinaux disparaissaient au 3ᵉ mois.

Des études récentes du contenu stomacal et intestinal et des selles de mangoustes de Hawaii ont montré que cet animal est omnivore et on a observé antérieurement que les mangoustes mangent les rats gisants, proches de la mort. L'examen du contenu de leurs selles donne un aperçu de la mortalité des rongeurs. Dans la vallée du Waipio, qui limite au nord-ouest la zone connue d'endémie pesteuse, 31 sérums de mangoustes ont été titrés: 9 (29%) réagissaient, 4 (12,9%) étaient positifs alors que sur 144 sérums de *Rattus exulans* examinés, 8 (5,5%) réagissaient, 2 (2,1%) étaient positifs. Dans des régions où la peste n'était pas suspectée, les résultats ont été semblables: dans le district de Kona, sur 77 sérums de mangoustes, 46 (59,7%) réagissaient, 18 (23,4%) étaient positifs; dans le district de Kohala, sur 51 sérums, 26 (51,1%) réagissaient, 8 étaient positifs, mais il ne fut trouvé de sérum réagissant que chez un seul rongeur. Les rares animaux capturés dans les districts de Kau et de Puna donnaient des résultats de même tendance. Les auteurs en concluent que la mangouste à Hawaii se comporte comme un hôte sélectif de *P. pestis* récolté à partir des rongeurs qui l'hébergent.

REFERENCES

Baltazard, M., Davis, D. H. S., Devignat, R., Girard, G., Gohar, M. A.. Kartman, L., Meyer, K. F., Parker, M. T., Pollitzer, R., Prince, F. M., Quan, S. F. & Wagle, P. (1956) *Bull. Wld Hlth Org.*, **14**, 457-509

Fourie, L. (1938) *S. Afr. med. J.*, **12**, 352-358

Gross, B. & Bonnet, D. D. (1951) *Publ. Hlth Rep. (Wash.)*, **66**, 1541

Meyer, K. F., Humphreys, F. A., Knapp, W., Larson, C. L., Pollitzer, R., Quan, S. F. & Thal, E. (1963) In: American Public Health Association, *Diagnostic procedures and reagents*, 4th ed., New York, chapter 13, p. 389

Mitchell, J. A. (1924) *Bull. Off. int. Hyg. publ.*, **16**, 566

Mott-Smith, E. (1910) *Annual report of the President of the Board of Health of Territory of Hawaii*, Honolulu, Bulletin Publishing Co., pp. 16, 107-113

Pirie, J. H. H. (1927) *Publ. S. Afr. Inst. med. Res.*, **3**, 119

Quan, S. F., Wheeler, C. M., Goldenberg, M. I. & Kartman, L. (1965) *Amer. J. trop. Med. Hyg.*, **14**, 638-642

Sever, J. L. (1962) *J. Immunol.*, **88**, 320-323

Simpson, W. J. R. (1905) *A treatise on plague*, Cambridge University Press, pp. 109, 115

Thornton, E. N. (1933) *Quart. Bull. Hlth Org. L.o.N.*, **2**, 64

Trainer, D. O., Lee, V. H. & Hanson, R. P. (1963) *Zoonoses Res.*, **2**, 105-116