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STUDIES ON PASTEURELLA PESTIS IN FLEAS*

Comparative Plague-Vector Efficiency of Xenopsylla vexabilis hawaiiensis and Xenopsylla cheopis

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SYNOPSIS

The authors report on a study carried out to determine the experimental plague-vector efficiency of *X. v. hawaiiensis* compared with *X. cheopis* after both species had been infected with a virulent Hawaiian plague strain (S113). In deriving the numerical values for vector efficiency the concepts of Wheeler and Douglas were followed with some modifications. An additional component, the blocking-survival potential, was used to obtain a vector index.

The experiments showed that the mean extrinsic incubation period was shorter in *X. v. hawaiiensis* than in *X. cheopis* but that the latter species produced more blocked fleas. The observed values for vector efficiency indicate that *X. cheopis* was about twice as efficient in plague transmission as the Hawaiian flea. The time and percentage mortality curves of mice dying of plague after blocked fleas had fed upon them were observed to be similar to the curves of mice succumbing to the intracutaneous inoculation of known dosages of *P. pestis*.

In general, it was found that bacteriological culture of the faecal droppings of fleas was unreliable as a check on plague infection in fleas.

The flea genus, *Xenopsylla*, is represented in the Hawaiian Islands by two known species, *X. vexabilis hawaiiensis* and *X. cheopis*. The latter species was probably introduced to the islands via domestic rats on ships. The former species is considered to be the only truly indigenous flea on the

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islands and was probably introduced prior to western exploration and settlement.

The presence of the Hawaiian flea was first demonstrated in April 1932 by Eskey [9] and the species was described as *X. hawaiiensis* by Jordan.[16] The specimens sent to Jordan were taken from the native Hawaiian rat, *Rattus hawaiiensis*, on the islands of Hawaii and Maui. In his original description, Jordan suggested that *X. hawaiiensis* was possibly a geographical variety of *X. vexabilis*, which he had formerly described [15] in 1925, from Franklin Island, South Australia. *X. hawaiiensis* was also shown to be similar to *X. nesiotes* from Christmas Island, *X. nubica* from Africa, and *X. astia* from Asia.

In a later paper (Jordan [17]), *X. hawaiiensis* was shown to be one of three subspecies, of which the other two were *X. vexabilis* and *X. meseris* from Queensland. Thus the Hawaiian flea became known as *X. vexabilis hawaiiensis*. However, Augustson,[2] after studying topotypic specimens of *X. hawaiiensis*, concluded that this was a synonym of *X. vexabilis*. Augustson apparently had failed to note Jordan's paper of 1936, but his discussion supports Jordan's contention that *X. hawaiiensis* is a subspecies of *X. vexabilis*. Modern taxonomic concepts support this contention since a subspecies is considered to be a geographically and/or a reproductively isolated portion of a species population which is in the process of independent evolution.

The foregoing taxonomic history has been given in some detail since it is important to establish the fact that the Hawaiian flea is not actually *X. vexabilis vexabilis* of Australia, but is a morphological entity which can be readily distinguished from its Australian relative. Thus its role as a vector of plague in the Hawaiian Islands is given a taxonomic distinction which should be generally known.

Eskey [9] suggested that plague in the Hawaiian Islands has consisted of (1) an urban type associated with domestic rats and *X. cheopis*, and (2) a rural type associated with feral domestic rats, the Hawaiian rat, and *X. cheopis* and *X. vexabilis hawaiiensis*. The urban type has been a self-limiting epidemic variety (Eskey ; [9] Link [20]), whereas the rural type is primarily enzootic in the Hamakua District of Hawaii Island and the Makawao District of Maui Island.

X. v. hawaiiensis is found chiefly on rats in the field (Eskey; [9] Kartman & Lonergan [19]), and its paramount role in maintaining enzootic and epizootic plague in field rats was postulated by Eskey [9] upon extensive epizootiological evidence. Thus, as shown by Kartman & Lonergan,[19] control of rural plague in the Hamakua District of Hawaii would depend mainly upon an attack on the field rat and the Hawaiian flea.

Information regarding the history of plague in Hawaii and its ecology and control may be found in the papers by Eskey,[9] Gross & Bonnet,[13] Link,[20] and Kartman & Lonergan.[18, 19]

In spite of rather extensive epidemiological and epizootiological observations, practically nothing is known in regard to the plague vector efficiency of *X. v. hawaiiensis*. Eskey [9] reported one positive experimental transmission of plague to white rats with 8 fleas, and 4 negative experiments in which from 3 to 5 fleas per trial were used. Beyond this, no experimental work appears to have been done to define the vector capabilities of the Hawaiian flea. Since this problem represents one of the critical unsolved questions of the epidemiology of plague in Hawaii, the following experiments were conducted to provide an experimental comparison of *X. v. hawaiiensis* with *X. cheopis* in regard to their comparative efficiency as hosts to, and vectors of, *Pasteurella pestis*.

Methods and Materials

Bacteriological procedures

A virulent strain of *P. pestis* (S113), originally obtained from a rat on Maui Island, Hawaii, in 1946, was used in these studies. The lyophilized culture was reconstituted with sterile distilled water and was inoculated intraperitoneally in 0.25 ml volumes into 3 white mice. The mice died after 2 days, and bacteria, recovered from the spleens, were inoculated into brain infusion broth and incubated at 28°C. After 24 hours, 0.05 ml of the broth culture was transferred to 5.0 ml of fresh broth and the remainder was tested for purity.

The culture resulting from the transfer was used in a 10^{-2} dilution to infect white mice by intraperitoneal inoculation of 0.5 ml per mouse. In 36 to 48 hours these mice exhibited a terminal septicaemia in which *P. pestis* abounded in the blood. Each of 4 mice in this condition was used to feed each sex per species of flea. Estimates of the number of *P. pestis* in the blood of these mice, determined on the basis of colony counts on blood-agar plates of samples taken before and after flea feedings, showed approximately from 10^8 to 10^9 bacteria per ml of blood for each of the 4 mice.

Only one mouse survived for more than half an hour after the fleas were placed on it, and it was sacrificed. This mouse had been used to feed the *X. cheopis* females. It is not known whether a possible difference of physiological activity of this mouse was a factor in the rate of infection of these fleas.

At intervals after the infectious feeding, 6 fleas per species per sex were taken at random to be used in *P. pestis* counts. These fleas were killed by exposing them, in their separate test tubes, to hydrocyanic acid gas. Each flea was then separately ground with a 0.2 ml aqueous suspension of 1% Attasorb with a pestle in a mortar, and then the volume was increased to 1.0 ml with 1% peptone water. The number of *P. pestis* present was determined by the conventional plate-count using blood-agar plates. The

results of these counts are summarized in Table I. The data suggest that the fleas, *X. v. hawaiiensis* and *X. cheopis*, infected with *P. pestis* strain S113, may show differences in their capacity to become infected and to eliminate bacteria after infection. Although the two species of fleas are approximately of the same size, a lower percentage of *X. cheopis* was

TABLE I. COUNTS OF "PASTEURELLA PESTIS" IN INDIVIDUAL FLEAS AT VARIOUS INTERVALS AFTER THE INFECTIOUS FEEDING ON WHITE MICE WITH TERMINAL SEPTICAEMIA

Flea species	Sex	Flea no.	All counts x 10^3 in descending order at intervals shown			
			2-4 hours	24 hours	48 hours	72 hours
X. v. hawaiiensis	M	1	250	500	600	400
		2	200	300	500	350
		3	200	100	500	150
		4	100	23	300	—
		5	30	8	0.3	—
		6	2	0.2	0.2	—
	F	1	1 500	500	600	1 000
		2	250	500	500	7
		3	200	60	12	5
		4	100	50	7	2
		5	75	30	2	0.003
		6	15	0	2	0.001
X. cheopis	M	1	250	0.2	1	250
		2	100	0	0.01	100
		3	15	0	0	100
		4	0	0	0	1
		5	0	0	0	0.001
		6	0	0	0	0
	F	1	1 000	54	60	100
		2	100	0.2	9	100
		3	10	0	0	20
		4	1	0	0	20
		5	1	0	0	0
		6	0	0	0	0

infected directly after feeding and the intensity of the infection also was less. Of those *X. cheopis* infected, the rate of decrease of the bacteria in the fleas after 24 hours was much higher than in *X. v. hawaiiensis*. By the third day, the bacterial count in the *X. cheopis* had increased to an order approximately equal to that at 2-4 hours after the infectious feeding. In general, there was an earlier appearance of the point of inflexion on the curve of the *P. pestis* counts in the male fleas.

Entomological procedures

The strain of *X. v. hawaiiensis* was from an insectary culture originally established in February 1954 with 4 specimens obtained from *Rattus hawaiiensis* in the plague-enzootic region of Hawaii Island. These were reared on white rats in an air-conditioned room which was maintained at a temperature of approximately 24°C and a relative humidity of 80%. Since this was the first time that this flea has been cultured *en masse*, the details of techniques and results will be published elsewhere (Stark & Kartman, in preparation).

The *X. cheopis* was from the strain which has been maintained at this laboratory during the past three years and originally established with fleas taken from domestic rats in San Francisco. They were reared under the same conditions as those described for *X. v. hawaiiensis*.

Twenty-three days prior to the initiation of the experiment, pupae of both species of fleas were screened from the sand in their culture containers and placed into separate jars which were kept in the insectarium. Two days before the experiment, the emerged adults were sexed by placing them on water in a pan, illuminating them with a strong light, and selecting the males and females by eye. Males and females were placed in separate glass containers with strips of paper towelling and kept in the insectarium.

Four mice, previously inoculated with plague and showing terminal septicaemia, were used to infect the fleas. Batches of from 50 to 100 fleas, per species per sex, were placed on the mice which were in jars placed in a large enamel pan. After the fleas had been given ample time to feed, the mice were removed from the jars and the fleas blown off them into the pan. All but one of the mice were dead when they were removed from the pan. The fleas were then aspirated into separate test tubes and again checked under a dissecting microscope with regard to sex. Each flea was then observed microscopically after placing it in a drop of water on a glass slide and covered with a circular 12 mm cover-slip. Only fully blooded fleas were retained.

On the 3rd and 4th days after the infectious feeding, each flea was allowed to feed on a clean mouse and was again examined microscopically. Only those fleas were retained which showed some visual evidence of possible infection, i.e., small dark masses or a cloudiness in the ventriculus. With

most of the fleas, an adequate number was found with which to carry on further observations. On the other hand, of 34 *X. cheopis* females retained, only 9 showed evidence of possible infection; of the remainder, 13 were cultured on blood-agar and only one of these proved positive for plague.

Each flea retained was kept in an individual test-tube. The test-tubes were in racks placed in a flea-proof structure previously used in experimental epizootic studies,[7] the storage temperature fluctuating from about 21°C to 23°C and the relative humidity from about 69% to 73%. All fleas were numbered separately and a separate record card was kept for each.

The fleas were given an opportunity to feed upon clean white mice at frequent intervals, usually every two or three days. The mice used were mainly 9- to 15-week-old male Namru No. 1 albino mice. Towards the end of the experiment male mice were not available and female mice of the same strain and age were used. Altogether, 237 males and 19 females were used. When used for feeding fleas, the mice were closely clipped on the ventral side and then confined to special plastic tube restrainers.[1] After the fleas had fed, the mice were kept in individual jars of the type described by Wheeler & Douglas.[27] The mice were observed for a period of 10 days and, if they had not died before then, were sacrificed, dissected, and cultured bacteriologically for *P. pestis*. Mice dying before 10 days were dissected, observed for gross plague lesions, and smears and cultures were made from the heart and the spleen. Details of the methods used at this laboratory in plague transmission studies have been published by Link & Prince.[20]

During the early feedings, when the visible evidence of apparent growth of the plague organisms had progressed only as far as ventricular or proventricular masses, or both, several fleas were fed on one mouse. Later, partially or fully blocked fleas were fed singly upon individual mice. After an opportunity to feed, each flea was examined microscopically to determine whether it had fed and observations were made in regard to the development of plague masses. Blocked fleas were usually given more than one opportunity to feed on several consecutive days or on the same day; each blocked flea was allowed to feed up to 30 minutes on a mouse. Data regarding the length of feeding of each flea, its microscopic appearance after feeding, date of death, results of bacteriological culture after death, and fate of the mouse were recorded. Sketches of the microscopic appearance of each flea were made after feeding and coloured photomicrographs were taken of some fleas. Thus the progress of the bacterial growth could be followed in individual fleas. After the death of a flea, it was triturated and cultured on a blood-agar plate and its faeces were cultured separately. On occasion, fleas were transferred to clean test-tubes and the faeces in the old test-tubes were cultured.

Data and Discussion

The ability of *P. pestis* to form a proventricular block in a particular flea species is probably dependent upon a complex of factors. This problem has been discussed in detail by Burroughs,[5] who discounted the theory of Blanc & Baltazard [4] that blockage depends upon the multiplication of the organisms in fresh blood taken up by the flea at frequent feedings. Burroughs [5] found that blockage was more rapid when the fleas had starved for several days. This is consistent with the general increase in the *P. pestis* count 2-3 days after infection (Table I) without further feeding. It was observed in the present experiments that a flea may be apparently blocked, but that in persistent attempts to feed this block may be broken by the flushing action of the blood as it is forced into the proventriculus. Thus, in the present report, a blocked flea is defined as one in which the proventricular block is not broken through after the flea has been allowed to feed. In this condition the flea usually shows the effects of starvation and desiccation by a decrease in size due to telescoping of the body segments. After attempts to feed, the oesophagus is quite often seen to be U-shaped, rather than comparatively straight (Faasch [12]), as it leaves the proventricular region and it is quite often enormously expanded.

The importance of temperature to the plague-vector capabilities of fleas has been discussed by Burroughs [5] who pointed out the lack of data on this problem. It may thus be of importance to indicate here that the temperature range under which these fleas were held in the laboratory (21°-23°C) is almost the same as the average variation in temperature of the plague-enzootic area around Honokaa, Hawaii (*Yearbook of Agriculture, 1941*), which is a natural habitat of *X. v. hawaiiensis*. *X. cheopis* is also found in this region.

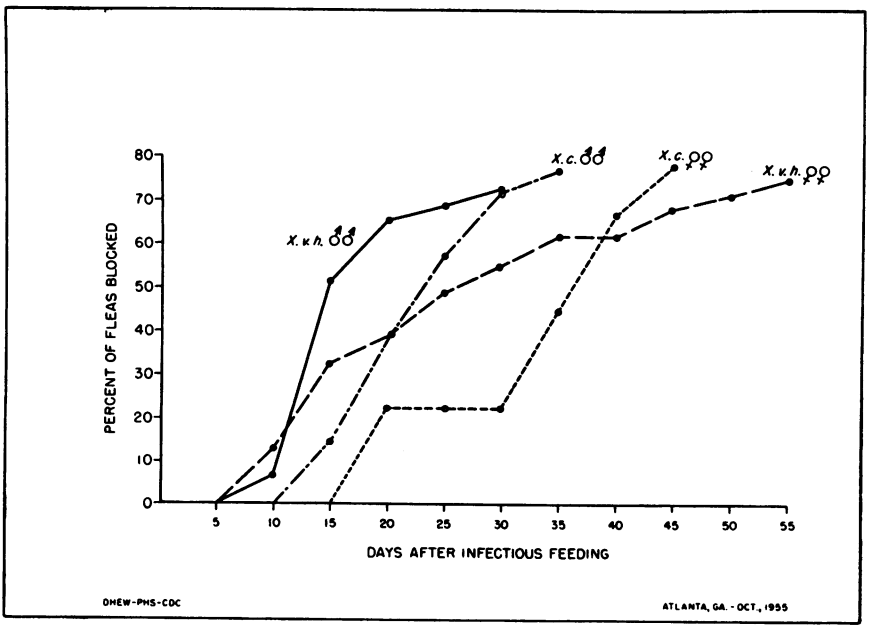
The frequency of blocking and the blocking rate of both flea species are shown in Table II and Fig. 1. Although the *X. v. hawaiiensis* blocked sooner than the *X. cheopis*, the latter species produced more blocked fleas than the former. For the *X. v. hawaiiensis* males, the shortest extrinsic incubation period (from infectious feeding to blocking) was 10 days, the longest 29 days, the mean 14.5 days, with a standard deviation (SD) of 6.08. For the females, the shortest period was 7 days, the longest 52 days, the mean 22.4 days, with an SD of 12.76. For the *X. cheopis* males, the shortest period was 13 days, the longest 31 days, the mean 20.3 days, with an SD of 5.09. For the females, the shortest period was 17 days, the longest 42 days, the mean 30.9 days, with an SD of 8.66.

In the studies by Eskey [10] on the blocking phenomena in various species of fleas, it was pointed out that in *X. cheopis* plague masses originated in the proventriculus more frequently than in the case of other species observed. In the present study the development of plague masses appeared

TABLE II. FREQUENCY OF BLOCKING IN FLEAS AFTER THE INFECTIOUS FEED

Number of days after infectious feeding	Number of fleas blocked				
	X. v. hawaiiensis		*X. cheopis*		Totals
	M	F	M	F	
0-4	0	0	0	0	0
5-9	0	3	0	0	3
10-14	12	7	3	0	22
15-19	6	1	5	2	14
20-24	2	4	4	0	10
25-29	1	2	3	0	6
30-34	0	2	1	2	5
35-39	0	0	0	2	2
40-44	0	2	0	1	3
45-49	0	1	0	0	1
50-54	0	1	0	0	1
Total blocked/ total examined	21/29	23/31	16/21	7/9	67/90

FIG. 1. CUMULATIVE BLOCKING-RATE OF FLEAS, INFECTED WITH "PASTEURELLA PESTIS", AT VARIOUS DAYS AFTER INFECTIOUS FEEDING



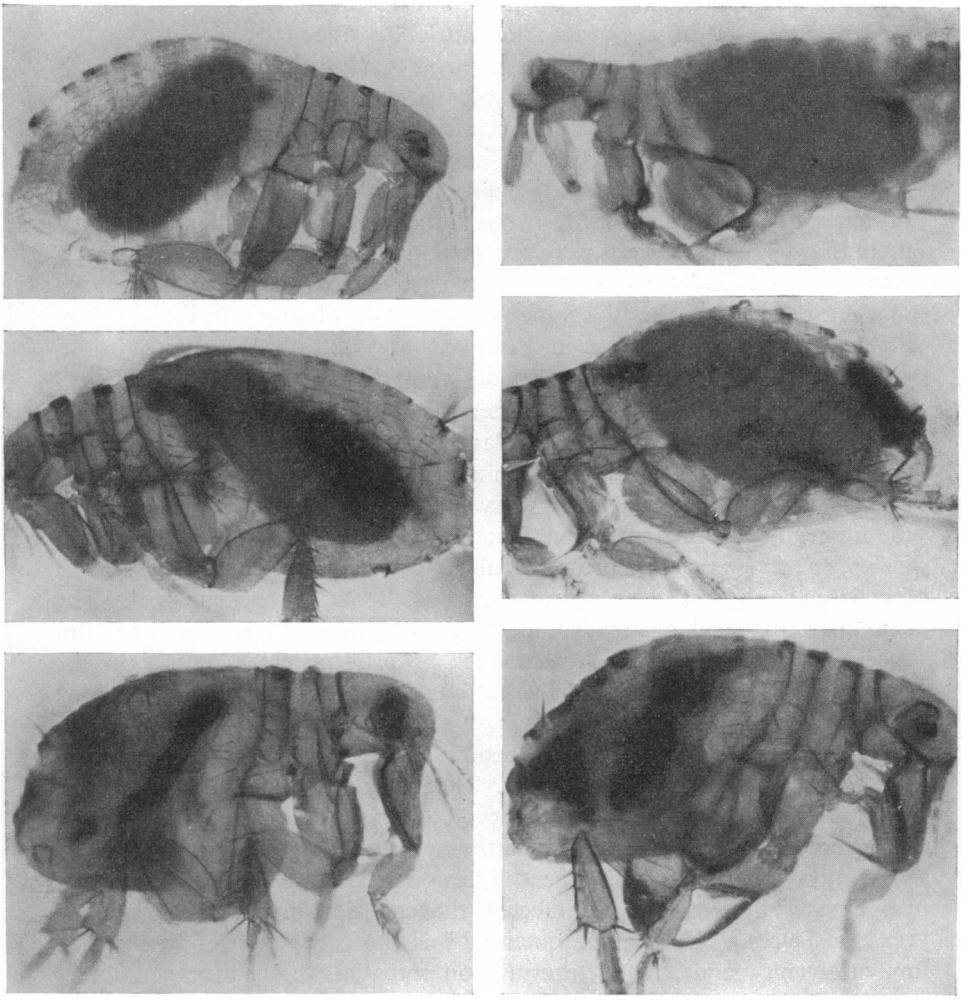
to be similar in the two flea species. Plate I illustrates some of the developmental stages of *P. pestis* in the fleas. The first observations on a possible development of plague masses were made between 3 and 5 days after the infectious feeding. For *X. v. hawaiiensis* males, plague masses were originally noted in the proventriculus in 6 fleas, and in the ventriculus in 18 fleas. For the females, the figures were: proventriculus, 6; and ventriculus, 23. For *X. cheopis* males, these figures were: proventriculus, 3; and ventriculus, 17. For the females the figures were: proventriculus, 1; and ventriculus, 7. All of these fleas did not retain their infections as can be seen in Table III, nevertheless the data show that the plague organisms developed similarly in both species and that ventricular masses predominated at the onset of observable masses. For *X. cheopis*, this contrasts with Eskey's statement that " no large masses were observed in the stomachs of *X. cheopis* ".[10] The use of a different plague strain may partially explain the predominance of ventricular masses as the initial phenomenon observed in the development of the Hawaiian strain in *X. cheopis* and *X. v. hawaiiensis*. Various strains of *P. pestis* are known to differ in their composition.[7, 8, 25]

At a later point—10-12 days after the infectious feeding—the picture presented by the development of plague masses in the fleas had changed considerably. For *X. v. hawaiiensis* males, plague masses were noted in the proventriculus in 19 fleas, and in the ventriculus in 4 fleas. For the females, the figures were : proventriculus, 14 ; and ventriculus, 12. For *X. cheopis* males, the figures were : proventriculus, 16; and ventriculus, 3. For the females, the figures were : proventriculus, 4; and ventriculus, 4. The similarity of the plague-mass findings in corresponding sexes of each flea species is striking. The predominance of proventricular masses in the males at 10-12 days is a further illustration of the greater rapidity of blocking in this sex under these conditions.

The primary objective of these experiments was to delineate the plague-vector efficiency of *X. v. hawaiiensis*. The method of individual flea transmission allowed the use of the concept of vector efficiency developed by Wheeler and Douglas.[28] These authors postulated three potentials for deriving vector efficiency. The " infection potential " is based on the percentage of fleas proved infected through demonstration of plague-positive faeces. The " vector potential " is the percentage of fleas which may transmit plague. The " transmission potential " is the average number of transmissions accomplished by the infective fleas when fed once daily on individual white mice. The " vector efficiency " is the product of these three factors and represents the number of transmissions effected by a given number of fleas.

In the present report, the infection potential is based on the percentage of fleas proved positive for plague after their death, since the criterion of positive flea faeces was considered to be unreliable in view of the findings of Burroughs [5] and as indicated in Tables V and VI (see page 702).

PLATE I. SOME OF THE DEVELOPMENTAL STAGES OF "PASTEURELLA PESTIS" IN "XENOPSYLLA CHEOPIS" AND "XENOPSYLLA VEXABILIS HAWAIIENSIS"



Xenopsylla cheopis. Top : female, immediately after the infectious blood meal. Middle: female, 8 days after infectious feeding showing proventricular plague mass with extension into anterior ventriculus and cloudiness in ventriculus. Bottom: female, 22 days after infectious feeding showing complete proventricular blockage; note blood in the oesophagus and solid plague mass in the ventriculus.

Xenopsylla vexabilis hawaiiensis. Top: male, immediately after the infectious blood meal. Middle: female, 8 days after infectious feeding showing proventricular plague mass with slight extension into ventriculus and cloudiness in ventriculus; note blood in oesophagus. Bottom: female, 9 days after the infectious feeding showing almost completely blocked proventriculus; note small amount of blood in the anterior ventriculus and the distended oesophagus filled with blood.

The vector potential is here termed the blocking potential and is based upon the percentage of fleas with permanent proventricular blocks. A flea which is apparently blocked and subsequently breaks the block would not be included until the blockage is permanently set. The transmission potential is the same as that defined by Wheeler and Douglas.[27]

In addition to the above components, another concept, the blocking-survival potential, has been included to derive a vector index. This potential is a ratio of the mean day of death of fleas after blocking to the mean day of blocking after the infectious meal (i.e., the duration of infectivity over the time taken to become infective). This potential is suggested inasmuch as speed of blocking and survival after blocking undoubtedly represent critical factors in the biological transmission of plague by fleas under natural conditions.[11, 23] The shorter the incubation period, and the longer the survival after blocking, the higher the blocking-survival potential. The vector index of the fleas is represented here as the product of the above four potentials.

The data on vector efficiency and the vector indices are summarized in Table III. It is interesting to note that for *X. cheopis*, originally obtained from San Francisco rats, the vector efficiencies based on transmission of plague to laboratory white mice were as follows : Wheeler and Douglas,[28] 0.43; Burroughs,[5] 0.66; the present study, 0.69. Eskey and Haas,[11] working with guinea-pigs, obtained data which give a vector efficiency of 0.19 for *X. cheopis*. However, their methods and the animals used do not allow a strict comparison with the work mentioned above. This illustrates the advantages of employing a standardized technique to compare vector efficiencies of different flea species.

Wheeler and Douglas [28] showed that the flea *Diamanus montanus* had a vector efficiency of 0.84, whereas *X. cheopis* had an efficiency of 0.43. They concluded that these differences indicated that *D. montanus* was nearly twice as efficient as *X. cheopis* as a vector of *P. pestis*. In the present experiment the vector efficiencies obtained appear to indicate that *X. cheopis* was about twice as efficient as a vector of plague as was *X. v. hawaiiensis*. An analysis of variance was applied to test the hypothesis that the ratios of plague transmission to total number of fleas used may have differed only because of chance. The results showed an F ratio of 4.95, indicating that for 1 and 88 degrees of freedom at the 5% level the disparity between the calculated variances was significant (the value of t was 2.224, $P=0.02$). Thus the calculated differences in the vector efficiencies of the two flea species are not due to chance but to some other factor or factors which have biological significance within the limits of the present experimental conditions. The effect of the blocking-survival potential can be seen in the vector index derived for the *X. cheopis* females (Table III). Since the females required a longer period to block and survived for a shorter period after blocking than the males, the female vector index is less than that of the males. In

TABLE III. SUMMARY DATA FOR THE DETERMINATION OF PLAGUE-VECTOR EFFICIENCY INDICES

Flea species	Sex	Number used	Number infected	Number blocked	Number of transmissions	Blocking-survival ratio [a]	Infection potential	Blocking potential	Transmission potential	Vector efficiency [b]	Blocking-survival potential	Vector index
X. v. hawaiiensis	M	29	23	21	9	3.5/14.5	0.79	0.91	0.42	0.30	0.24	0.07
	F	31	28	23	11	3.3/22.4	0.90	0.82	0.47	0.35	0.14	0.05
	M + F	60	51	44	20	3.4/18.9	0.85	0.86	0.45	0.33	0.17	0.05
X. cheopis	M	21	21	16	12	3.9/20.3	1.00	0.76	0.75	0.57	0.18	0.11
	F	9	8	7	9	3.0/30.9	0.88	0.87	1.28	0.99	0.09	0.09
	M + F	30	29	23	21	3.7/23.5	0.96	0.79	0.91	0.69	0.15	0.10

[a] Mean day of death after blocking/mean day of blocking after infectious meal
[b] Vector efficiencies calculated according to the method of Wheeler & Douglas [27]

the derivation of vector efficiency, however, the average transmissions by the females is higher than the value for the male *X. cheopis*. But this impression of over-all better efficiency by the females is not necessarily true, as shown by the vector indices.

It is suggested that the use of the blocking-survival potential allows a more complete analysis in the simultaneous evaluation of factors which affect the likelihood that a given flea species is an efficient vector of plague.

The frequency of plague transmission and the relation of number of transmissions to opportunities to transmit, by blocked fleas of both species, are shown in Table IV. For the present experiment, an opportunity to transmit plague is based upon the observation that a blocked flea had endeavoured to feed upon a susceptible mouse and that blood was seen in the oesophagus at the termination of the feeding attempt. The data in Table IV show that the ratios of number of transmissions to opportunities to transmit (transmission success) are considerably higher for the *X. cheopis* than for the *X. v. hawaiiensis*. The ratios for combined sexes of these species were, respectively, 0.36 and 0.18. Thus, as in the vector efficiency values, *X. cheopis* appeared to be twice as successful in plague transmission as was *X. v. hawaiiensis*.

TABLE IV. FREQUENCY OF PLAGUE TRANSMISSION BY BLOCKED FLEAS

Flea species	Sex	Number of fleas blocked	Number of fleas transmitting plague	Frequency of transmission			Transmission success [a]	Ratio
				1	2	3		
X. v. hawaiiensis	M	21	9	9	0	0	9/46	0.19
	F	23	7	4	2	1	11/59	0.18
X. cheopis	M	16	8	5	2	1	12/39	0.30
	F	7	5	2	2	1	9/18	0.50

[a] Number of transmissions/opportunities to transmit

It should be emphasized that the " true " vector efficiency of a flea species is necessarily dependent upon a complex of bio-physical factors in nature which are, at present, but poorly understood. Although a certain amount of work has been done on the microclimate of rodent burrows, on correlations of climatic factors with plague, and on the physiology and habits of fleas, there has been no attempt to synthesize existing information as a background for the evaluation of the plague-vector capabilities of a particular flea species under defined ecological conditions. Until this is done, experimental determination of the plague-vector efficiency of fleas will have to serve, at best, as a rough index.

The epizootiological implications of the vector-efficiency data suggest that, in the plague-enzootic region of Hawaii, *X. v. hawaiiensis* is an efficient vector of plague to rats, but not as efficient as is *X. cheopis*. Nevertheless, it should be indicated that, in the plague-enzootic region of the Island of Hawaii, *X. v. hawaiiensis* is the predominant flea affecting *Rattus hawaiiensis* and other species of rats in the field.[9, 19] Thus wild-rodent plague on the Hamakua coast is probably maintained by *X. v. hawaiiensis* because of three factors, its predominance in the field, its efficiency as a vector, and its prevalence on the Hawaiian rat which is thought to be essential to the maintenance of plague in the field.[9] The possibility also exists that the San Francisco *X. cheopis* used in these experiments and the *X. cheopis* from the Hamakua coast of Hawaii, where the *X. v. hawaiiensis* were obtained, may differ in regard to plague-vector efficiency. This type of geographical strain difference in a single flea species was shown to be statistically significant in regard to the flea *D. montanus*.[5]

Plague transmission was attained by fleas without proventricular blocks in only 3 cases. Seven days after the infectious meal, plague was transmitted to a mouse on which 30 *X. v. hawaiiensis* males had fed singly during one day. Of these 30 fleas only one was observed to have a proventricular mass and this flea might have been responsible for the transmission. In another instance, 10 days after the infectious feeding, plague was transmitted to a mouse upon which 21 *X. cheopis* males had fed singly during one day. Six of these fleas showed proventricular masses. In none of these cases did any of the fleas require more than 3-4 minutes to feed, which is typical of fleas having no proventricular obstruction. Thus these transmissions were independent of blockage and proceeded through a different mechanism.[5, 24]

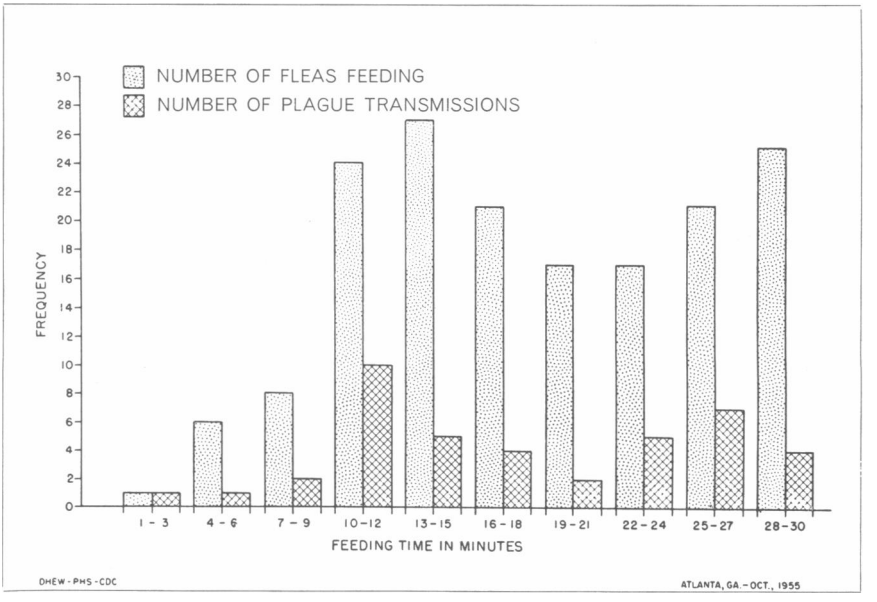
X. v. hawaiiensis female No. 13 transmitted plague 35 days after its infectious feeding and showed a proventricular mass with a finger-like extension into the ventriculus after the infective feeding. This flea died on the 36th day without becoming blocked, and its culture resulted in a confluent growth of plague on the blood-agar plate. *X. v. hawaiiensis* female No. 3 transmitted plague 14 days after the infectious feeding and showed proventricular and ventricular masses at the time. This flea later became blocked on the 20th day and transmitted plague on that day and also on the 21st day. The length of the infective feeding of these two fleas was, respectively, 5 minutes and 20 minutes. These two cases of plague transmission by partially blocked fleas illustrate the proventricular valve disorder described by Bacot [3] and also mentioned by Burroughs.[5] An interesting case is that of *X. v. hawaiiensis* female No. 5 which showed complete blocking on the 11th, 12th, and 14th days after the infectious feeding. On the 17th day it was given an opportunity to feed and it remained upon the mouse for 30 minutes in one position. During this time its apparently violent efforts to feed resulted in a ruptured oesophagus and blood was

forced into the haemocoele. Plague transmission resulted from this feeding, and the flea died on the 19th day.

None of the *X. cheopis* fleas with partial blocks transmitted successfully. In two instances only did mice die in which plague could not be confirmed. This occurred after feedings by a blocked *X. v. hawaiiensis* male and a blocked *X. cheopis* female. All of the plague transmissions occurred in the male mice, of which 237 were used. None of the 19 female mice succumbed although, in 11 instances, blocked fleas were fed upon them. This is not considered to be significant, however, since male and female mice of the strain used have been shown to be equally susceptible to plague in controlled experiments in this laboratory.

Table IV shows that many blocked fleas did not transmit plague, although they were given many opportunities to do so as shown by the presence of red blood in their oesophagi on microscopical examination after attempts to feed. This phenomenon has been amply documented in the works of Eskey & Haas,[11] Wheeler & Douglas,[28] Burroughs,[5] and Holden-ried.[14] In nature, blocked fleas have an opportunity to feed *ad libitum* and undoubtedly remain on the host for extended periods. A normal flea, or one without any obstructions in its intestinal tract, usually engorged fully within 3 to 4 minutes in this experiment. Blocked fleas were allowed to feed up to a maximum of about 30 minutes and then were removed from

FIG. 2. RELATION OF LENGTH OF FEEDING BY BLOCKED FLEAS TO PLAGUE TRANSMISSION



the mouse. It is possible that a greater number of transmissions may have resulted if the blocked fleas had been allowed to feed for much longer periods.

Data in regard to this problem have been summarized graphically in Fig. 2. These data are based upon the total feeding time of the fleas without regard to whether the feeding was multiple or in a single spot. The data show that, although some blocked fleas fed only for short periods, the tendency was for the fleas to feed for longer intervals. Many fleas had to be forcibly removed from the mouse. The data, however, do not suggest any definite correlation between the length of the feeding and plague transmission. If much longer feeding periods had been allowed, it may be possible that a positive correlation would have appeared and that the present data would merely represent the negative side of the comparison.

The question of single or multiple attempts by blocked fleas to feed is of interest here since Eskey & Haas [11] found that with infective fleas " . . . 64 per cent of their subsequent blocked feedings were infectious when the parasites made multiple attempts to obtain blood while only 28 per cent transmissions occurred when the insects ceased to feed after one bite ". In the present instance it was found that with blocked *X. v. hawaiiensis* males, 8 of the mouse transmissions were made after multiple feedings, whereas 1 of the transmissions followed a single feeding. On the other hand, with the *X. v. hawaiiensis* females, 2 of the transmissions followed multiple feedings and 9 resulted from single feedings. With the *X. cheopis* males the transmissions were equally divided, i.e., 6 resulting from multiple and 6 from single feedings. In the case of *X. cheopis* females, multiple feedings resulted in 6 transmissions, whereas single feedings produced 3 of the transmissions. These data generally corroborate the findings of Eskey & Haas,[11] but they suggest that the phenomenon is not a consistent one as is shown in the case of *X. v. hawaiiensis* females. It is possible that the number of bites taken by any particular blocked flea may be influenced by factors induced by the artificial conditions under which fleas are experimentally fed. For instance, when mice are restrained in plastic holding-tubes, wire tubes, or in other ways for upwards of from 15 to 30 minutes, their occasional struggles disturb fleas feeding upon them. Some blocked fleas have been seen to remain in one position in spite of vigorous movements by the mouse, whereas others are immediately disturbed and move to another position. The continuous activity of the rodent host in nature may stimulate similar patterns of feeding by blocked fleas. Thus the data on multiple or single feedings are undoubtedly representative of such influences and of others which have not been adduced. Accordingly, laboratory data of this type are merely suggestive and their relation to the actual conditions in nature remains unknown.

In microscopic observations of individual blocked fleas after feeding it was noted that the oesophagus presented differences in appearance

which changed from the first feeding after blocking to the last feeding before death of the flea. In most cases this was manifested by an enormous distention of the distal oesophagus during later feedings and it was thought that this represented more violent efforts of the flea to feed which might result in more successful plague transmission. The data for blocked fleas of both species with regard to the frequency of a particular feeding as related to transmission are summarized as follows :

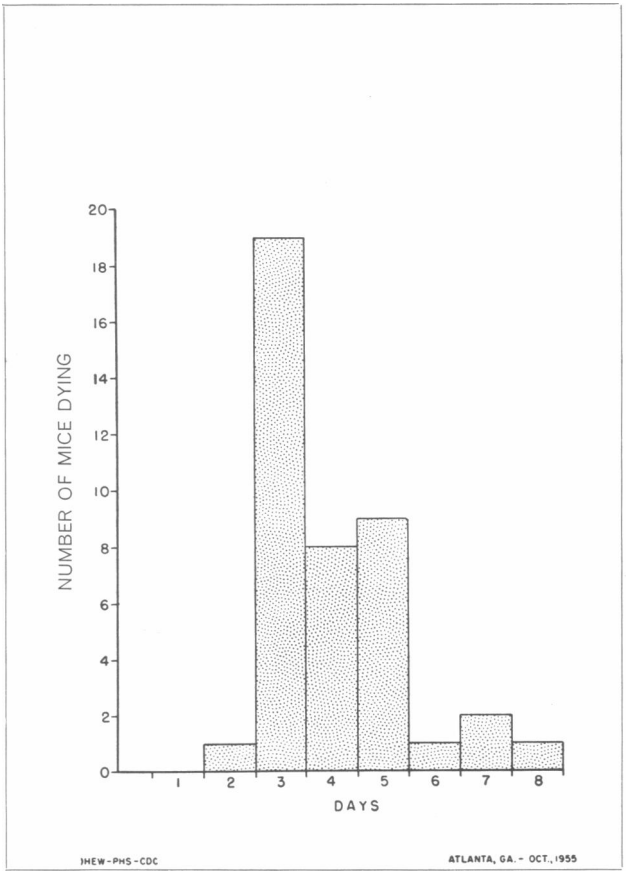
Feeding	*Number of feedings*	*Number of transmissions*
1st	67	15 (22%)
2nd	55	12 (22%)
3rd	28	10 (36%)
4th	10	3 (30%)
5th	1	0
6th	1	1
	162	41

Since fleas die in a very few days after blocking (see below), the number of fleas able to feed after their third or fourth feeding would be rather small even though they might be offered a meal every day. The data given above illustrate this decline in the frequency of fleas feeding for the fifth or sixth time after blocking, but there appears to be no significant trend in the number of plague transmissions. Analysis of the species and the sexes separately showed similar results. Up to and including the fourth feeding the percentages of transmissions are remarkably close. The importance of the figures for the fifth and sixth feedings may be discounted because of the small number of cases involved.

Of 20 mouse deaths following plague-infective feedings of *X. v. hawaiiensis*, 9 were caused by male and 11 by female fleas. The mean life of these mice after infection was 4.1 and 4.5 days respectively. Of 21 mouse deaths following plague-infective feedings of *X. cheopis*, 12 were caused by male and 9 by female fleas. The mean post-infection life of these mice was 3.4 and 4.0 days respectively. The mean day of death of all these mice was 4.0. Fig. 3 shows the frequency of mice succumbing to plague infection by fleas.

Quan (unpublished observations) has been able to demonstrate differences in the slopes of the time and percentage mortality curves in relation to known dosages of intracutaneous inoculation of mice with *P. pestis*. Fig. 4 presents such curves compared with curves of mouse deaths after flea transmission. Using the method of Litchfield,[22] the ET_{50} is the time when 50% of the mice would be dead. In the present data, for *X. cheopis* the ET_{50} is 2.7 (2.3-3.2) days with a standard deviation (SD) of 1.5 (1.3-1.7); and for *X. v. hawaiiensis* the ET_{50} is 3.6 (3.0-4.2) days with an SD of 1.4

FIG. 3. FREQUENCY OF MICE SUCCUMBING TO PLAGUE TRANSMITTED BY BLOCKED FLEAS AT VARIOUS DAYS AFTER INFECTIVE BITE

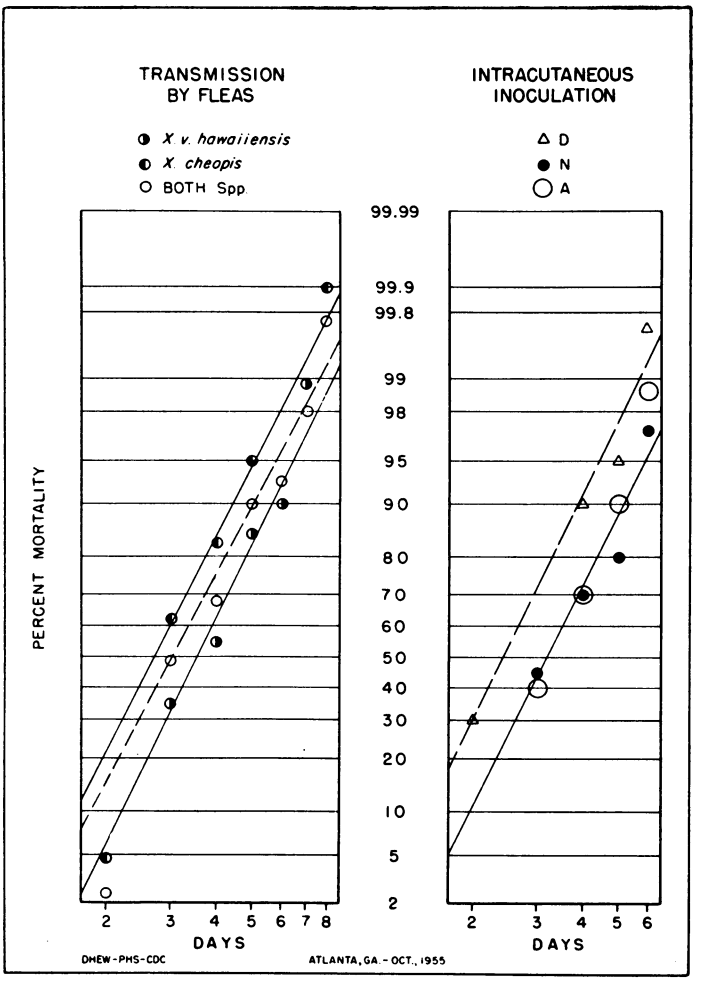


(1.3-1.6). The combined ET_{50} is 3.0 (2.7-3.4) days with an SD of 1.5 (1.4-1.6).

Similar calculations have been made for the 10^{-3} dilution used with the same strain of mice, but with a different plague strain (New Mexico human strain). In experiments N and A (Fig. 4) the actual number of *P. pestis* evaluated by plate-counts was 350 and 200 respectively. The ET_{50} was 3.2 (2.8-3.6) days with an SD of 1.4 (1.3-1.6). In experiment D, when 400 organisms in the 10^{-3} dilution were inoculated into the Webster strain of Swiss mice, the ET_{50} was 2.4 (2.0-2.9) days with an SD of 1.5 (1.3-1.6).

Although no significant quantitative differences have been reported for the infectivity of different virulent strains of *P. pestis*, it is known that different strains of mice respond to infection at a different rate. In the

FIG. 4. PERCENTAGE MORTALITY, BY INTERVAL FROM INFECTION, OF MICE INFECTED WITH "PASTEURELLA PESTIS" AFTER INFECTIVE FLEA BITES AND AFTER INTRACUTANEOUS INOCULATIONS OF 0.05 ML SUSPENSIONS OF PLAGUE ORGANISMS



X. v. hawaiiensis. $ET_{50} = 3.6$
X. cheopis. $ET_{50} = 2.7$
Combined. $ET_{50} = 3.0$

D. dose = 400 *P. pestis*, $ET_{50} = 2.4$
N. dose = 350 *P. pestis*, $ET_{50} = 3.2$
A. dose = 200 *P. pestis*, $ET_{50} = 3.2$

present experiment, there is a definite resemblance of the time and percentage mortality curves of mice dying of plague from flea infection and those dying after intracutaneous inoculation of 0.05 ml of an aqueous suspension containing approximately 3×10^2 virulent *P. pestis*. The above comparison is naturally a rough one and has been made with a number of assumptions which still need experimental verification. It is of interest that the number

of organisms estimated here is far below that which has been previously suggested (Burroughs [5]) as the probable number regurgitated by blocked fleas during their infective feeding.

Although other workers have mentioned the importance of flea survival in plague transmission experiments, Holdenried [14] stated that the survival-rate under the conditions of the experiment was important in the proper evaluation of plague-vector efficiencies. He presented a table showing the survival of fleas after plague-infectious feedings. Wheeler & Douglas [28] charted the frequency of plague transmission and the longevity of *X. cheopis* and *D. montanus*. In the present report, the blocking-survival relation has been used in the evaluation of vector efficiency (see Table III).

FIG. 5. SURVIVAL-RATES OF FLEAS AFTER BLOCKING WITH "PASTEURELLA PESTIS"

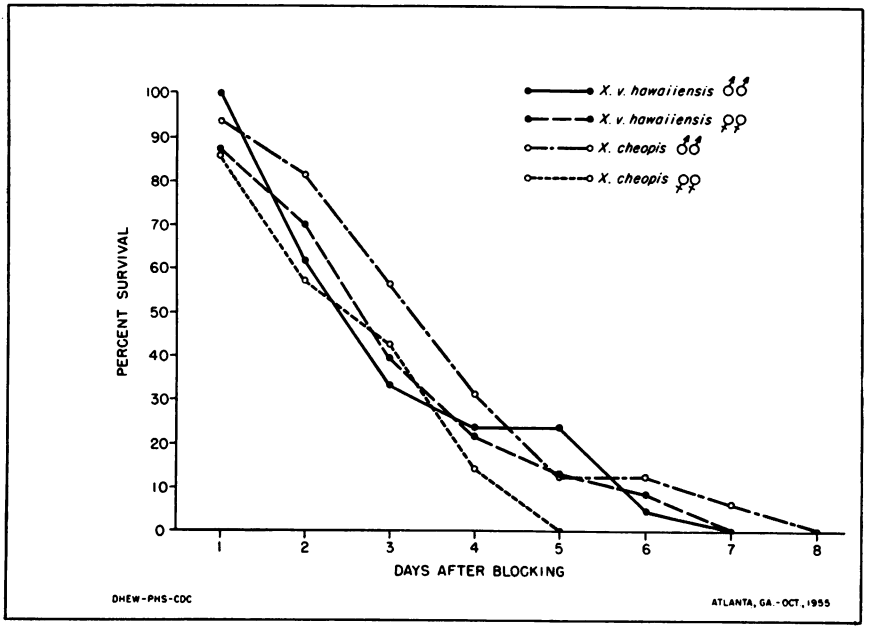


Fig. 5 presents a graphic illustration of the survival-rate of fleas after permanent blocking with *P. pestis*. The survival-rate in both sexes per species appears similar. The minimum and maximum number of days that the fleas lived after blocking were respectively: *X. v. hawaiiensis* males, 2 and 7 (mean 3.4); *X. v. hawaiiensis* females, 1 and 7 (mean 3.3); *X. cheopis* males, 1 and 8 (mean 3.9); *X. cheopis* females, 1 and 5 (mean 3.0). Of fleas that blocked, the life span, from the infectious feeding to death, averaged 25 days. Fleas that did not block lived an average of 32 days, and

7 fleas (2 *X. v. hawaiiensis* males and 4 females; 1 *X. cheopis* female) which had never shown any plague development lived for 60 days then they were sacrificed in good condition.

Burroughs [5] has suggested that the failure of captive fleas to feed regularly may be the chief factor contributing to early death. In the present experiment the fleas were given the following number of opportunities to feed, the number of refusals to feed being given in parentheses: *X. v. hawaiiensis* males, 222 (33); females, 281 (64); *X. cheopis* males, 144 (18); and females, 96 (21). These figures include all fleas, whether blocked, infected, or uninfected and suggest that the fleas took an adequate number of blood meals for proper nourishment.

Burroughs [6] has indicated that the longest lived *X. cheopis* in the laboratory were those fed daily when kept at 20°C and 92% relative humidity. In this laboratory, the experience has been that *X. cheopis* and other flea species will usually refuse to feed one day after a satiation feeding. Burroughs [6] has also shown that unfed fleas, either kept singly or together, at a similar temperature and humidity to that in the present experiment, had a median survival time of from 6 to 9 days. This laboratory has had similar experience with unfed *X. cheopis* and the survival time is considerably above that of fleas after blocking with *P. pestis*. This suggests that the blocking phenomenon may exert more than a mere mechanical effect upon the flea and that the rapidity of death after blocking may be due to other factors in addition to starvation and desiccation. Additional studies on this problem are needed with special reference to temperature and humidity.

It may be of interest to note that 6 *X. v. hawaiiensis* females, not included in the main transmission experiment, were kept singly and unfed in test-tubes under the same laboratory conditions. On the 19th day after the infectious feeding they were all allowed to feed and one showed a complete proventricular block and died on the 20th day, and 2 others showed partial blocks and died on the 20th and 21st days. All of these were positive for plague on culture but none transmitted plague. The remaining 3 fleas showed no evidence of blockage or plague masses and were negative for plague on culture. One died on the 21st day, one on the 31st day, and one on the 58th day.

Faecal droppings in test-tubes, from which fleas had been removed, were cultured. For blocked fleas, these droppings represented faeces deposited before the flea had blocked and thus were several days old at the time of culture. Also, at several times during the experiment, fleas were placed in new test-tubes and the accumulated faeces in the old test-tubes were cultured. Summaries of these data are presented in Tables V and VI. The general unreliability of faecal culture in the determination of plague-infected fleas shown by these data corroborates the findings of Burroughs [5] and Holdenried.[14] Although there appears to be a perfect correlation between the microscopic appearance of apparently plague-free fleas and

TABLE V. BACTERIOLOGICAL PLAGUE FINDINGS IN FLEAS AFTER DEATH

Flea species	Sex	Number examined	Blocked fleas			Fleas with plague masses			Fleas apparently clear		
			number	% positive	% faeces positive	number	% positive	% faeces positive	number	% positive	% faeces positive
X. v. hawaiiensis	M	29	21	100	57	2	100	100	6	0	0
	F	31	23	100	57	6	83	50	2	0	0
	M + F	60	44	100	57	8	88	63	8	0	0
X. cheopis	M	21	16	100	63	5	100	60	0	—	—
	F	9	7	100	71	1	100	100	1	0	0
	M + F	30	23	100	65	6	100	67	1	0	0

TABLE VI. SUMMARY OF ALL CULTURES OF FLEA FAECES AT VARIOUS TIMES AFTER THE PLAGUE-INFECTIOUS FEEDING

Flea species	Sex	6 days		18 days		33 days		55 days	
		number of specimens	% positive	number of specimens	% positive	number of specimens	% positive	number of specimens	% positive
X. v. hawaiiensis	M	29	17	24	67	5	20	3	0
	F	31	32	31	55	12	83	4	25
	M+F	60	25	55	60	17	65	7	14
X. cheopis	M	—	—	21	86	2	100	—	—
	F	—	—	8	75	6	100	—	—
	M+F	—	—	29	83	8	100	—	—

the results of culture, the numbers available are too small for any definite statement. In the present experiment, it has been noted that a visual criterion of " clear " is not always a reliable diagnosis of the absence of infection in the flea since many individual fleas without visible masses in the proventriculus or ventriculus have been shown to be plague-infected on culture. This was especially true of *X. v. hawaiiensis.*

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RÉSUMÉ

On ignorait encore le rôle respectif que jouent dans la transmission et le maintien de la peste à Hawaï les deux espèces de puces vectrices connues dans cette île: *Xenopsylla cheopis*, considérée comme indigène, et *X. vexabilis hawaiiensis*, variété géographique de *X. variabilis* décrite d'après des spécimens d'Australie du Sud, importée probablement avec les rats par voie maritime.

Les auteurs ont tenté de résoudre ce problème épidémiologique, d'une importance capitale. Ils ont cherché à déterminer expérimentalement l'importance relative de ces deux espèces de puces, d'après divers facteurs et indices — les uns déjà utilisés par certains chercheurs, d'autres modifiés ou nouveaux.

Les techniques délicates qu'ils ont appliquées à l'élevage des puces, à l'infection des souris sur lesquelles se nourrissaient ces puces, à la culture des broyats de puces pour évaluer le nombre de bacilles pesteux dont elles étaient porteuses, ainsi que l'analyse statistique des résultats, sont exposées de façon détaillée.

Les auteurs considèrent comme « potentiel d'infection » le pourcentage de puces infectées par le bacille pesteux. Ils font intervenir le phénomène de « blocage » dans la notion de « pouvoir vecteur potentiel », qui devient dans cette étude « potentiel de blocage ». Ce dernier est calculé d'après le pourcentage de puces dont le proventricule est bloqué de façon irréversible par l'accumulation des bacilles pesteux, qui empêche la transmission et entraîne la mort des puces. Le « potentiel de transmission » est le nombre moyen de transmissions à des souris sensibles effectuées par les puces nourries une fois par jour sur les souris infectées.

Un « indice vecteur » a été en outre introduit par les auteurs dans ces déterminations. Cet indice est calculé d'après le potentiel de survie après blocage; il est exprimé par le rapport suivant: nombre de jours de survie après blocage sur le nombre de jours entre le repas infectieux et le blocage. Cet indice peut être d'un grand intérêt, car les facteurs

sur lesquels il est fondé interviennent de façon déterminante dans la transmission de la peste dans la nature.

D'après les facteurs considérés dans cette étude, il semble que *X. cheopis* soit un vecteur deux fois plus actif que *X. v. hawaiiensis*. Mais il faut rappeler ici que l'importance du rôle vecteur d'une espèce dans la nature dépend d'un complexe bio-physique encore mal exploré. Tant que n'est pas mieux connue l'influence du climat, du microclimat des terriers et de divers phénomènes physiologiques sur le pouvoir vecteur des puces, il faudra s'en tenir aux résultats des recherches expérimentales pour l'évaluer.

Les données expérimentales indiquent que, dans la région d'enzootie pesteuse d'Hawaï, *X. v. hawaiiensis* est un vecteur actif de la peste du rat, moins important cependant que *X. cheopis*. Toutefois, il convient de souligner que, dans la région d'endémie pesteuse d'Hawaï, *X. v. hawaiiensis* est la principale espèce de puce vivant sur *Rattus hawaiiensis* et d'autres espèces de rats des champs. Il est probable que la peste est maintenue par cette puce dans la région d'Hamakua, en raison de son efficacité comme vecteur et de sa fréquence sur *Rattus hawaiiensis*, qui doit jouer un rôle essentiel dans le maintien de l'enzootie.

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