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The rôle of the infected and the infective flea in the spread of sylvatic plague.

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Renewed interest in the epidemiology of both rodent and human *P. pestis* infections on the North American Continent followed the recognition of widely distributed foci of sylvatic plague in California and various other Western States. It was noteworthy and the subject of spirited discussion that despite the existence of these active reservoirs with hundreds of infected rodents, very few cases of human plague were diagnosed. Even if it is admitted that some transmissions to man may have escaped detection on account of their indefinite characteristics, the total number of actual human plague cases during the past 3 years does not exceed 10 (1935-1, 1936-5, 1937-2). However, these unfortunate, terror striking, accidental infections occured quite unexpectedly during the summer in recreational areas. What was even more disconcerting was the fact that the anatomical examination of the rodents shot and trapped in the same region yielded negative findings. Thus the source and the spread of the plague bacillus was shrouded in a discomforting mystery. At this stage it was recalled that KITASATO[1]) and the Indian *Plague Research Commission* [2]) and in particular SWELLENGREBEL and HOESEN [3]) had found many years ago that in instances where infected rats were scarce or apparently altogether absent, it

[1]) Lancet, 1894, **2**, p. 428.

[2]) Jour. Hyg., 1906, **6**, p. 421; 1907, **7**, pp. 323, 693; 1908, **8**, p. 161; 1910, **10**, p. 313, Plague Supplements I—V, 1911—1917, p. 1—899.

[3]) Zeitschr. f. Hyg. u. Infektionskrankh., 1915, **79**, p. 436.

was comparatively easy to collect plague-infected fleas for adequate examination. Furthermore, the problem of latent plague without visible lesions and the brilliant studies of S. M. NIKANOROV[4]) and his pupils on sylvatic plague in Russia, thus far ignored in the American plague studies, received consideration. Beginning in 1936, routinely the gross anatomical examination of the different rodents *(Citellus* varieties, *Eutamias*, *Cynomys*, *Glaucomys*, *Peromyscus*, *Neotoma* and *Marmota)* were supplemented by collections of fleas killed with chloroform [5]). The insects were sent to the laboratory in salt solution. There they were washed in sterile salt solution and ground in a mortar. The suspensions were then injected subcutaneoulsy into guinea pigs. Since 1937 the spleen and lymph-nodes of the various rodents have been assembled in pools and have been tested by subcutaneous injection on guinea pigs.

These surveys embracing in 1936 14 080 fleas and 6 812 rodents and in 1937 nearly 104 000 fleas collected from 30 332 squirrels, ground-hogs, chipmunks, mice, etc. revealed the existence of plague-infected fleas on animals which had anatomically been declared as non-infected. The significant data are summarized in Table I. In certain regions the demonstration of fleas carrying the plague bacillus was the only evidence that the disease was in operation. Even more intriguing was the fact that infected fleas were found on rodents removed from burrows proven to have harbored at least 20 years ago diseased and anatomically infected squirrels. The existence of clandestine foci of plague was indeed a new discovery. Finally, one observation created considerable interest. In the course of the survey activities incident to a human case of plague, 10 fleas had been collected from the bedsheets in a summer cottage located in the vicinity of the Lake Resort where the case had through fleabites contracted the disease. The ground insects produced typical plague on inoculation of a guinea pig. A persual of the odd 55 case histories dealing with plague infections of supposed squirrel origin fail to report fleabites in about 40. Although it is a matter of conjecture as to the actual number of cases which were caused by fleabites, the evidence covering the last 3 years strongly incriminates direct contact with the wild rodents and not transmission through insects. Both the early reports by WM. B. WEAVER [6]) and the more recent

[4]) Rev. Microbiol. and Epidemiol., 1924, **3**, p. 224; Rongeurs et Puces dans la conservation et la transmission de la Peste, Paris, 1928, p. 96.

[5]) K. F. MEYER, Am. Jour. Pub. Health, 1937, **27**, p. 777.

[6]) Jour. Infect. Dis., 1908, **5**, p. 485.

Table I.

Summary of positive examination of fleas in California and Nevada.

Counties	1936				1937			
	Number of Fleas	Collected from Rodents	Total Number of Pools	Infected Pools	Number of Fleas	Collected from Rodents	Total Number of Pools	Infected Pools
El Dorado	—	—	—	—	7,675	5,143	204	1
Fresno	—	—	—	—	2,041	1,322	48	6
Modoc	1,675	2,104	67	5	—	—	—	—
Monterey	2,759	733	41	1	13,248	3,827	109	0
Placer	416	153	23	3	3,411	3,537	71	1
San Benito	285	122	2	1	4,491	511	14	0
San Bernardino	1,480	483	42	2	3,966	1,776	68	11
San Mateo	18,729	802	155	6	3,825	74	35	0
Santa Cruz	2,154	1,350	35	7	5,345	789	20	0
Douglas, Nevada	—	—	—	—	208	168	38	1

observations [5]) emphasize the fact that the handling of sick or dead rodents, the skinning of squirrels and their preparation for food, occasional bites and similar accidents preceded the appearance of bubonic or carbuncular human lesions. On the other hand, squirrel hunters and the members of the sylvatic plague survey crews are known to have been covered and even severely bitten by sqirrel fleas without any consequences. These and many other paradoxical observations demand an explanation since they are not in harmony with the views held by many that the vectors carrying plague bacilli are regularly capable of transmitting the parasite. A discussion of this problem requires a consideration of both the flea and the mammalian host. For the sake of clarity, it is advisable to treat the subjects historically.

Through the studies of Ogata[7]) in Formosa in 1897 and then Simond[8]) in 1898, the rôle of the flea in the spread of plague was established experimentally. Although Verjbitzki[9]), Thompson[10]) Liston[11]) and others showed the presence of plague bacilli in the intestinal tract of fleas, it was reserved for the Indian Plague Research Commission[2]) to investigate the paramount importance of the rat flea, *Xenopsylla cheopis*, in the transmission of the infection both from rat to rat and from rat to man. As possible modes of transfer of the infective agent, they considered the following:

(a) Mechanical conveyance of the plague bacilli by the pricking apparatus. (b) Ingestion of infected fleas by the animal. (c) Regurgitation of the stomach contents or retention of infected blood about the mouth parts or in the pharynx, the bacilli being either injected with the saliva, carried down on the epipharynx or rubbed into the prick wound. (d) Rubbing or injection of faeces deposited by the flea into the wound.

The Commission layed stress on the wound infection by faeces. However, in 1913 Swellengrebel[12]), working in Java, showed that *Xenopsylla cheopis* did not defecate when feeding and that the faeces were very sticky; the skin was less likely to be soiled in this way. By feeding infected fleas on guinea pigs, he succeeded in producing plague in guinea pigs. Swellengrebel also concluded

[7]) Zentralbl. f. Bakteriol., 1897, **21**, p. 769.
[8]) Ann. de l'Inst. Pasteur, 1898, **12**, p. 625.
[9]) Jour. Hyg., 1908, **8**, p. 162.
[10]) Jour. Hyg., 1906, **6**, p. 537.
[11]) Jour. Bombay Nat. History Soc., 1905, **16**, p. 253.
[12]) Rep. Nederl.-Ind. Civil Med. Serv., 1913, p. 1.

that the bacilli were introduced by the proboscis of the fleas. The frequency of defecation varies with the species of fleas and the individual insects (NÖLLER[13]) and ESKEY[14])). It remained for BACOT and MARTIN[15]) in 1914 to elucidate the mechanism of the infective process leading to the transmission of the *P. pestis* by the flea. They demonstrated that the multiplication of the bacilli in the stomach may ultimately lead to blockage of the proventriculus. Such "blocked" fleas become highly infective as the oesophagus contains virulent bacilli which quite regularly infect any fresh blood sucked in. A "blocked" flea makes frantic efforts to suck in blood since it is unable to get nourishment. A cessation of these efforts leads to an elastic recoil of the pharyngeal and stomach walls, which drives the infected blood back into the bite wound. In a partially "blocked" flea a column of infected blood extends from the stomach to the anterior chamber of the pharyngeal pump and thus renders the insect bite particularly dangerous. In their experiments relatively few and probably "low virulent" bacilli passed in the faeces of the fleas, and consequently BACOT and MARTIN argued that infection by means of faecal droppings was entirely adventitious. These experiments and observations were made on *Xenopsylla cheopis* and *Nosopsyllus fasciatus*, and it was shown that *Xenopsylla cheopis* was a more efficient vector than *Nosopsyllus fasciatus*. These facts have again been emphasized by ESKEY[14]). Although epidemiological data from South Wales, Queensland, Tasmania and Hawaiian Islands fully attest to the vital rôle of *Xenopsylla cheopis* in the propagation of bubonic plague, it is well to remember that MITAL[16]) found little or no correlation between the percentage figures of *Xenopsylla cheopis* and the annual plague rates in India. In fact, the old observations by the Plague Research Commission in India showed that out of 67 fleas collected from rats and exposed individually on healthy guinea pigs, only 1 actually transmitted the infection. In 1915 SWELLENGREBEL and HOESEN[3]), while studying plague during interepidemic months, made the important discovery that in 18 instances the presence of plague was demonstrated by means of the inoculation of material from 15,279 triturated fleas taken from 3,230 rats. No human cases of plague were observed during the same

[13]) Arch. f. Protistenk., 1912, **25**, p. 386.

[14]) Pub. Health Rep., 1938, **53**, p. 49.

[15]) Jour. Hyg., 1914, **13**, Plague Suppl. III., p. 423.

[16]) Rongeurs et Puces, Paris, 1928, p. 183.

period. More recently GEORGE and WEBSTER[17]), in the course of their inquiry into plague in the Cumbum Valley in South India call attention to the important difference between infected (pestiferous) and infective (pestigenous) fleas. The mere sucking of blood carrying *P. pestis* by the flea does not necessarily render it infective i. e. likely to transmit infection. Even under the most favorable conditions only an occasional infected flea becomes blocked and capable of infecting. Neither the total number of fleas found on the rodents, not even the number of infected fleas but the number of infective fleas is of prime importance. The physiologic-pathologic processes, which lead to the blocking of fleas, are greatly influenced by climatic factors.

Doubtless similar observations have been made by GIRARD[18]) in Madagascar, who tries to explain the demonstration of infected fleas on healthy rats by assuming the presence of a bacteriophage either in the vector or in the rodent.

The problem concerning infected and infective fleas has primarily been studied in connection with the vectors found on rats. The pertinent question arises: Are the facts applicable to sylvatic plague? In order to answer it one must search the extensive field and laboratory studies published by the Russian workers in articles not readily accessible. One realizes that the evidence—in particular the vector efficiency—regarding the fleas is by no means as well worked out as that concerning their hosts. Each rodent has its own hordes of fleas and the rôle as vectors must be analyzed individually. The studies of GOLOV and IOFF (1925—1927)[19]) are in this connection quite important. These two Russian workers experimented in detail with the fleas *(Oropsylla silantiewi, Ceratophyllus tesquorum* and *Neopsylla setosa)* of the Siberian marmot *(Citellus pygmaeus)* and the pulicidae *(Ceratophyllus mokrzeckii* and *Ceratophyllus consimilis)* of the field mouse *(Mus musculus* and *Musculus Wagneri).* Although nearly all types of fleas were capable of transferring plague from rodent to rodent when used in large numbers, in one test as few as 3 insects infected the experimental animals. Less successful was BYTCHOV[20]) who fed 624 fleas collected from

[17]) Indian Jour. Med. Res., 1934, **22**, p. 77.

[18]) Comp. rend. Soc. de biol., 1935, **120**, p. 133.

[19]) Rep. 1st All Russian Antiplague Conf. Saratov, 1927, pp. 102 and 158; Rev. Microbiol. and Epidemiol., 1926, **5**, No. 4.

[20]) Parasites, transmitteurs, animaux venimaux, Rec. Travaux dédié au 25me anniversaire scientifique du Professeur EUGÈNE PAVLOVSKY, Moscow, 1935, p. 89.

tarbagans, marmots and rat-hares *(Ochotona daurica)* on different experimentally infected rodents. In 61 individual experiments using as many as 40 fleas, not one transmission succeeded. He emphasizes the fact that transfer of the plague virus will not take place until at least a partial blocking of the proventriculus and ventriculus of the flea has taken place. In fact, a wild rodent flea infects quite irregularly either immediately after sucking infected blood or later when massive growth of the plague bacilli leads to obstruction of the proventriculus. Already in 1928 BYTCHOV and BORZONOV[21]) by using a cultural method in seeding the intestinal tube of dissected fleas on agar noted that normal insects with no signs of "blocking" of the stomach yielded positive cultures. TUMANSKI and POLIAK[22]) investigated 1,797 fleas recovered from a marmot nest in November; 5 fleas yielded *P. pestis* although none was blocked. Based on these and similar observations the Russian workers list the various modes of transmission of plague from rodent to rodent in the following order of importance:

(1) Crushing of infected fleas with the teeth, infection through the mucosa of the buccal cavity with subsequent involvement of the lymph-nodes of the neck.

(2) Scratching and rubbing of infected fleas, rarely faecal droppings into superficial skin wounds or abrasions.

(3) Through the bite of the flea with a soiled proboscis following the sucking of blood from a diseased rodent or

(4) later as an infective-blocked insect.

Analyzing the epidemiology of sylvatic plague in its relation to man, the observers in Saratov and Trans-Baikal conclude that immediate contact with the sick or dead rodent leads to bubonic plague, while flea transmission is infrequent. The danger represented by individual fleas, therefore, appears more limited than was originally believed. Aside from serving as vectors, the wild rodent fleas act, according to the observations of GOLOV and IOFF[19]) TUMANSKI and POLIAK[22]) EVSEEVA and FIRSOV[23]) as "preservers" of the plague infection in the burrows. Thus infected fleas obtaining an occasional meal may be alive for many months particularly in regions where the moisture of the environment is high but the temperature is low. Systematic studies concerning the influence of the "microclimate"[24])

[21]) Rev. Microbiol. and Epidemiol., 1929, **8**, Nr. 1.

[22]) Rev. Microbiol. and Epidemiol., 1931, **10**, p. 325.

[23]) Rev. Microbiol. and Epidemiol., 1932, **11**, p. 281.

[24]) See BUXTON, P. A., Tr, Roy. Soc. Trop. Med. and Hyg., 1933, **26**, p. 325.

in the burrows both on the preservation and in particular in the development of infective fleas from merely infected insects have not been made. Without a thorough understanding of the ecology of the species of fleas infecting the different rodents, it is impossible to evaluate the vector efficiency and the present day risk of sylvatic plague to man. However, from the available information, it is clearly demonstrated that the problem of infected versus infective flea applies also to the pulicidae of the wild rodents. In all probability, the vector efficiency plays an important rôle as has been pointed out by ESKEY [14]).

In considering the rôle of the infected flea, one important observation by BYTCHOV [20]) must not be overlooked. Eight guinea pigs used in the feeding experiments with plague infected fleas discussed in the preceding paragraphs survived when again bitten by 10-40 plague fleas; 1 month later all 8 animals proved immune against a dose of *P. pestis* fatal to controls. These observations and those by the Indian Plague Research Commission on rats suggest to him the realization that also under natural conditions rodents bitten by infected fleas may not succumb but become actively immunized. This hypothesis deserves experimental investigation for the following reasons:

a) Although the flea with a stomach capacity of 0.5 cmm. of blood may ingest at least 5 bacilli from a rat with 10 000 plague bacilli per c. c. in the blood stream. No estimates concerning the number of bacilli which are regurgitated from a "blocked" flea are available. In fact, it is reasonable to surmise that the viscous consistency of the "block" may contribute a variable number of *P. pestis* to the inoculum. Of interest are, in this connection, the findings of BORZONOV[21]) who bacterioscopically counted 70 112 and 1 026 921 plague bacilli, respectively, in 3 infected fleas. There is no standard method of testing virulence except the procedure by SOKHEY on mice. At least 12 to 24 plague bacilli are required to cause an infection by the intraperitoneal route. Probably a considerably larger number is needed to infect by the cutaneaus route and, futhermore, the infective dose may vary with the species of rodent. Finally, the ability of the flea to become infected will depend on the number of plague bacilli in the blood stream of the sick or dying rodents. Nothing definite is known regarding this phase of the problem. Feeding of wild rodent fleas on plague-infected guinea pigs may not imitate the conditions as they occur in the burrows.

b) With increasing frequency, attention has been called by SWELLENGREBEL and HOESEN [3]), WILLIAM and KEMMERER [25]) KONSTANSOFF [26]), NIKANOROV [27]), GÄISKY [28]), MEYER and EDDIE [29]) and others to latent invisible plague in various rodents. It is not unlikely that the transmission through infected fleas plays an important rôle in creating latency or even immunity. It is remotely possible that the non-blocked but infected flea may encourage the selective survival of low or even avirulent bacilli which are excellent antigens (OTTEN)[30]).

Finally, an attempt may be made to outline as a working hypothesis the epidemiology of sylvatic plague.

Migrating rodents, probably representatives of the Muridae, transport infected and infective fleas into the colonies of squirrels or chipmunks. A variable number of the Sciuridae will succumb to acute plague, thus contributing to the creation of an infected flea population of variable numerical strength. Shortly before hibernation, some of the rodents will develop latent or localized non-fatal plague. An altered metabolism of the squirrels, the predominance of infected fleas with plague bacilli of a lowered infectiousness and many unknown factors contribute to these conditions. Rodents with latent infections will hibernate only to develop acute plague early in Spring (March and April). Since the flea population is, as a rule, simultaneously very high, a great reservoir of infected vectors is thus created. The cadavers of the dead rodents are rapidly and effectively removed by the larvae of the *Lucilia*-flies, while the fleas persist in the nests. With the migration of the young squirrels and chipmunks into the empty abandoned burrows and nests, highly susceptible hosts are thus brought in contact with infected and infective fleas. They may bring the vectors to the surface and some may thus contribute to the intensity and the expansion of the plague virus. These events are probably accompanied by a variable degree of subclinical immunization favored by factors of age, and reduced

[25]) Pub. Health Rep., 1923, **38**, p. 1873.

[26]) Zentralbl. f. Bakteriol., I. Abt., Orig., 1933, **127**, p. 440.

[27]) Seuchenbekämpfung d. Infektionskr., 1927, **4**, p. 140.

[28]) Proc. 5th Antiplague Congress, U. R. S. S., Saratov, p. 258; Rev. Microbiol. and Epidemiol., 1926, **5**, No. 1; Arch. biol. nauk., 1926, **26**, No. 1.

[29]) California and West. Med., 1935, **43**, p. 399 and Am. Jour. Pub. Health, 1937, **27**, p. 777.

[30]) Geneesk. Tijdschr. Nederl.-Indie, 1932, **72**, p. 281 and Jour. Hyg., 1932, **32**, p. 396.

metabolism due to the approaching hibernation. Again latent infections and infected fleas in the burrows furnish the chain which connects the epidemics of one year with that of the next. Thus sylvatic plague smolders for years and is everlasting. Suppressive measures against sylvatic plague in order to be effective must by necessity be directed against the host—the rodents—and the vectors—the various species of fleas. In selecting the procedures to reduce the rodent population chemicals, preferably gases which are also insecticidal, must be chosen.