SYLVATIC PLAGUE STUDIES

THE VECTOR EFFICIENCY OF NINE SPECIES OF FLEAS COMPARED WITH *XENOPSYLLA CHEOPIS*

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(With Plates 4 and 5)

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INTRODUCTION

The discovery of the existence of a large wild-rodent reservoir of plague in the western United States during the last forty years stimulated interest in the study of the vectors infecting these rodent populations. Since the time that vector studies were first inaugurated, numerous investigators have proved that fleas are the only important plague vectors—that although other arthropods may be capable of becoming infected, their role in the transmission of the disease is incidental. And, further, it has been repeatedly illustrated that while some species of fleas are particularly efficient vectors, others possess few or none of the characteristics of the good vector.

The greatest amount of work has been done with fleas of the genus *Xenopsylla*, particularly with the species *cheopis*.

Russian workers, as well as American, having become aware of the widespread existence of sylvatic plague in the steppes, valleys, foothills and mountains encompassing thousands upon thousands of square miles, undertook a study of the vector ability of the flea fauna of various rodents known to be subject to periodic plague epizootics. These investigations, correlated with ecological research, have helped to explain the seasonal rise and fall of epizootics, the carry-over of plague from one season to another, and have disclosed the animal populations most susceptible to plague and the species of wild rodent-infesting fleas most hazardous to man.

The tendency during the past two decades has been to make comparative studies of different species of wild-rodent fleas in relation to their ability to transmit plague. Similar work has been

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done with rat fleas. Several species of fleas native to North America are now known to possess varying abilities as vectors.

American workers have recently presented a method which enables one to obtain the vector efficiency of a given species (with regard to biological transmission in contrast to mechanical or faecal transmission) and compare it with the vector efficiency of other species.

A definite aim has been made in the present studies to determine the practicability of using this method for a comparative study of the different species of fleas which occur in western North America. The primary purpose of the studies is an attempt to cast further light on the epizootiology of sylvatic plague. It is hoped that this purpose has been realized.

HISTORICAL

The etiological agent of plague was discovered during an outbreak of the disease at Hongkong at the close of the nineteenth century by Yersin (1894), a French scientist, who gave a good description of the organism. Kitasato (1894), a Japanese worker, also announced the discovery of an organism in plague cases, but presented an erroneous description. According to Lagrange (1926), Kitasato is to be honoured for having publicly stated that Yersin alone was the discoverer of the plague bacillus. Roux (1897) first expressed the view that plague was primarily a disease of rats. About the same time, Ogata (1897) concluded, on epidemiological grounds, that the rat is the principal host and the flea the means of dissemination of the causal organism in the rat population. He succeeded in producing plague in laboratory animals by inoculating them with triturated fleas taken from dead rats. Ogata was suspicious of fleas, as they were frequently the most numerous ectoparasites of rats, and were known to leave dead rats in search of a blood meal.

Simond (1898), after having made successful mass-transmission experiments, reported the transmission of plague from rat to rat by fleas. He showed that the cannibalistic and necrophilic habits of rats rarely resulted in their infection, and that enormous numbers of bacteria had to be ingested before such infections could occur. He concluded that the pathology then differed from that of animals infected by the cutaneous route.

Simond, furthermore, attached significance to the local reaction which appears in about 5 % of human plague cases. This local reaction manifests itself as a blister-like eruption and contains many plague bacilli. He logically concluded that fleas, which frequently attack the lower extremities where the reaction was found, were probably responsible. Experimentally, Simond found that plague-infected rats free from parasites were unable to transmit the disease to healthy rats kept in the same cage. However, if fleas were present, transmission from sick to healthy rats usually did occur. Simond thought that flea faeces, which he had found to contain plague organisms, might be a means of percutaneous infection. His early observations even extended to a possible explanation of the carry-over of plague from one year to another, for he found that fleas could remain infected for a considerable period.

Simond's excellent work was repeatedly confirmed in the next few years by various investigators, although his theories did not fail to arouse criticism. Thompson (1903) supported Simond, as did Gauthier & Raybaud (1902–3), who obtained several positive results in mass-transmission experiments with rats and rat fleas.

To the problems already presented by the existence of plague in rats were added questions brought about by the discovery of a large wild-rodent reservoir of plague in Asia, North America, South America and Africa. Numerous species of wild rodents were known to be involved, and each species was frequently found to harbour one or more species of fleas. The ability of the various species of fleas to serve as vectors of plague presented an important problem. Clemow (1900) reported some of the earliest isolations of rodent plague in animals other than domestic rats. He stated that bacteriological proof existed of the occurrence of spontaneous plague in monkeys, bandicoots, mice and grey-striped squirrels. The following corrected list of animals found naturally infected with plague was taken from a table presented by Wu & Pollitzer (1932):

Tarabagan (*Arctomys* [*Marmota*] *sibirica*),* Transbaikalia and Mongolia, 1895.

Squirrel (*Sciurus* [*Funambulus*] *palmarum*), Ceylon, India, 1898.

Mole rate (*Gunomys* [*Bandicota*] *bengalensis*), Ceylon, India, 1906.

Striped mouse (*Rhabdomys pumilio*), South Africa, 1906.

Hamster rat (*Cricetomys gambianus*), South Africa, 1906.

California ground squirrel (*Citellus beecheyi*), California, 1908.

Dusky-footed wood rat (*Neotoma fuscipes*), California, 1910.

Tree rat (*Epimys* [*Rattus*] *dolichurus*),† East Africa, 1913.

Small suslik (*Citellus pygmaeus*), South-east Russia, 1913.

* Wu & Pollitzer listed this as *Arctomys bobac*. This species occurs in Poland, while *sibirica* occurs in Transbaikalia.

† No reference to this species could be found.

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Large suslik (*Citellus fulvus*), South-east Russia, 1917.

Multimammate mouse (*Rattus coucha*), South Africa, 1921.

The world-wide sylvatic reservoir of plague proved to be of epidemiological significance. Wu (1934) described an outbreak first reported in 1907 in south-east Russian Turkestan. A Kirghese caught a black marmot and skinned it. He became sick soon afterwards, and a localized epidemic of pneumonic plague, affecting forty-six persons, subsequently occurred. Wherry (1908) stated that Past Assistant Surgeon Rupert Blue, as early as 1903, became impressed with the possibility of ground squirrels carrying the infection, upon noting that case histories of plague patients indicated contact with them. Human infection acquired while handling ground squirrels was reported by McCoy & Wherry (1909).

The importance of the wild-rodent source of plague has been repeatedly emphasized. Thornton (1933) states that in the small inland outbreaks of plague which occurred in South Africa in 1907, 1912 and 1914, no concurrent rodent epizootic was observed among domestic rodents, which were few in number or entirely absent. In 1921 definite proof was forthcoming that the veld rodents were infected (see Mitchell, 1927). Gerbilles and multimammate mice, dead of plague, were found in the vicinity where a farm worker had contracted the infection. The part played by these animals in the epidemiology of plague in rural South Africa is now well proved. The multimammate mouse, which readily seeks a domestic environment, carries the disease from gerbilles in the wild to man in the domestic situation.

Discussing the possible origin of plague in Africa, Meyer (1942) states: 'In trying to explain the original source of the infection of the rodents of the inland districts of South Africa, Mitchell and Thornton advanced the view that the spread was brought about by a gradual extension through the coastal belt of the striped mouse epizootics, which were noted on the outskirts of the coastal towns during the outbreaks of the period 1900–5. During the Anglo-Boer War, remount and supply depots were established at various inland centres to which were railed supplies from infected ports. It therefore appears to Fourie (1938) that the gerbilles and multimammate mice became infected directly from rats in the inland depots rather than by a slow extension of the disease among striped mice from distant foci at the coast. Irrespective of these interpretations of the available facts, the original source of the wild rodent plague infection in the interior of South Africa must remain a matter of surmise.'

Many instances of human plague infection are known to have resulted from contact with wild animals, usually rodents. De la Barrera (1939) reported an outbreak of plague in rural Argentina in which the 'cuis' or fence rabbit was incriminated as the rodent reservoir. Meyer (1943) states that domestic rodents are the animals primarily responsible for bringing the disease into contact with humans. In a later article (1946), the same author cites two instances (1910–11 and 1919–20) of pneumonic plague epidemics in Manchuria which developed in an area where a large percentage of the population was actively engaged in trapping and skinning marmots and was, furthermore, housed in crowded underground inns. Meyer states that the wild-rodent reservoir is not the common source of human infection and that plague is ordinarily spread to man by domestic rodents. Human contact with the sylvatic plague reservoir has resulted in only sixty-six known plague infections in the United States, according to Meyer.

Verjbitski (1908) showed that *Pasteurella pestis* multiplies in the alimentary canal of fleas. Pursuing mass transmission studies, this investigator succeeded in transmitting the infection to rats with small lots of fleas. He obtained transmissions with as few as five fleas, but not with fewer, and concluded that infection was unlikely to occur from the bites of less than five fleas. Verjbitski fed infected lots of fleas on individual rats daily for 6 days, each lot on a new rat, and secured positive results from the feedings on the first to third days. He erroneously concluded that fleas were incapable of infecting animals later than 3 days after they had taken an infectious meal.

Liston (1924) stated that sections he made in 1903 of fleas fixed after an infectious blood meal showed many plague bacilli in the proventriculus, but that it remained for Bacot & Martin (1914) to discover the mechanism of transmission by blocking.

The Indian Plague Commission (1906) confirmed transmission from rat to rat with rat fleas. These workers in 1907 published a long series of findings containing what they considered to be experimental evidence of seasonal influences on the ability of fleas to transmit plague. During the epidemic season the percentage of positive results obtained in the laboratory was six times greater than in the non-epidemic period. These workers, by dissection, demonstrated that approximately 50 % of the fleas taken from rats dead of plague harboured the plague bacilli. However, only one of sixty-seven fleas which were removed from rats dead of plague and placed individually on white rats transmitted the infection. The Commission concluded that transference of infection by a single flea was only remotely possible.

A total of twenty-seven transmission experiments made with the cat flea, *Ctenocephalides felis* (Bouche,

1835), were all negative. Three of thirty-eight experiments with *Pulex irritans* Linnaeus, 1758 gave positive results, and two attempts with *Nosopsyllus fasciatus* (Bosc., 1801) were successful. The Indian Plague Commission (1907) suggested several possible ways in which fleas might transmit plague: (1) 'By the animal eating the infected fleas.' They regarded this method as of little importance, since infection by ingestion requires enormous numbers of bacilli, and also because in 70 % of these cases, the animals developed a primary mesenteric bubo. Of several hundred animals infected by fleas in the laboratory in no case was a mesenteric bubo discovered. (2) 'By the proboscis of the fleas mechanically conveying the bacilli from the infected to the healthy animal.' They considered it significant that the largest number of infections occurred when using fleas which had fed upon infected rats within 36 hr. before transference to normal rats. However, they could not reconcile the theory of mechanical transmission with the fact that infectivity sometimes continued for several days, during which time the flea had fed only upon healthy animals. (3) 'By a regurgitation of the stomach contents through the oesophagus and the pharynx, the bacilli being then injected with the saliva or on the pricker or being rubbed into the wounds made by the pricker.' It is interesting to note that blocking was not known until 7 years later. The possibility that regurgitated matter might find its way into the aspiratory canal formed by the epipharynx and the mandibles and pass down that channel was thus given consideration, although they lacked any evidence to prove the theory. (4) Still another method was given consideration: 'By the bacilli contained in the faeces being deposited on the skin and then being either injected by the pricker or rubbed into the wounds made by the pricker.' They called attention to the fact that some species of fleas, while feeding, deposit undigested blood, often quite copiously. They also showed that sufficient organisms were contained in the faeces of infected fleas to infect guinea-pigs when injected subcutaneously or when introduced by the cutaneous method.

By 1907 considerable work had been done on fleas as vectors of plague. Some questions had been answered, but many more remained unanswered.

The classical work of Bacot & Martin (1914) demonstrated beyond doubt that fleas are biological vectors of plague, and that mechanical and faecal transmissions are not the only means of establishing an infection in an animal. These workers, using fleas which were proved to be plague-infected through demonstration of the organism in faecal specimens, fed individual fleas on the shaved abdomens of rats, then observed the insects under a hand-lens. Certain fleas, they noted, sucked energetically and persistently, yet no blood entered the stomach; the oesophagus, however, became unusually distinct. Upon dissecting some of these fleas the proventriculi were observed to be blocked with what proved to be a solid mass of plague bacilli, and the oesophagi were more or less distended with blood. These investigators stated that though blood could not enter the stomach, this did not prevent the insect from sucking, as the pump which aspirates blood is in the pharynx. The flea suffering from thirst, no matter how persistent in its efforts to satisfy this appetite, only succeeds in distending the oesophagus. The blood in the distended oesophagus may flow out again on cessation of the sucking act. Bacot & Martin observed drops of blood to escape from the mouthparts of blocked fleas when the probosci were withdrawn.

Results of a study of longitudinal sections of blocked fleas were published by Bacot in 1915. He called attention to the clearance of a passage through the centre of a proventricular plug, and pointed out that such a rupture of the obstructing mass or organisms does not restore the valvular function of the proventriculus. This passage merely allows blood to flow out of the stomach as freely as it enters; in this condition the possibility of transmission is increased. Apparently, the first independent confirmation of blockage in fleas was made by Golov & Ioff (1925), who observed regurgitation of blood from the probosci of *Rostropsylla* (*Citellophilus*) *tesquorum* (Wagner, 1898) (= *Ceratophyllus tesquorum*), indicating that not only rat fleas exhibited the phenomenon.

Probably to Hirst (1925*a*) belongs the credit for the observation that not all fleas are equally efficient vectors. This author suggested as early as 1913 that the plague-free districts of the Madras Presidency in India perhaps owed their immunity to the prevalence of *Xenopsylla astia* (Rothschild, 1911) instead of *X. cheopis* (Rothschild, 1903), the flea most prevalent in plague areas. He later (1923, 1925*b*) supported this theory with experimental evidence which tended to show that *X. astia* is a relatively less efficient vector of plague than is *X. cheopis*. Hirst (1925*b*) showed that *X. cheopis* will transmit at temperatures at which *X. astia* is unable to act as a vector.

The Indian Plague Commission (1908) reported that the advance of a plague epidemic is checked when the mean daily temperature passes above 80° F. Brooks (1917) studied the influence of saturation deficiency and of temperature range on the course of epidemic plague in India. He concluded that plague epidemics cease when the temperature exceeds 80° F., if accompanied by a saturation deficiency of over 0·3 in., and that each range of temperature has a corresponding critical saturation deficiency.

Goyle (1927) supported Hirst's observations with experimental evidence. Mass transmission experiments carried out under identical conditions produced twenty-five transmissions with *X. cheopis* and only nine with *X. astia*, in fifty-two attempts with each species. The author suggested caution in the interpretation of these data as such results may not be obtained under natural conditions. In a later publication Goyle (1928) demonstrated that meteorological conditions exert a very real effect on the transmission capacity of fleas. *X. cheopis* was found not to transmit at a saturation deficiency of 0·6 in. accompanied by a temperature of 68° F., while 0·3 in. in saturation deficiency sufficed to check *X. astia*.

Evseeva & Firsov (1932) state that in 1925 and 1926 Golov & Ioff conducted many experiments to determine the length of time that plague bacilli are conserved in the living flea. The longest time recorded by these workers was 396 days at a temperature range varying from 0 to 15° C., with a relative humidity of approximately 90 %. Evseeva & Firsov buried infected fleas at different depths in ground-squirrel nests and left them over the winter. All living fleas recovered were dissected and the stomach contents cultured bacteriologically. A few fleas in these experiments remained alive and infected throughout the winter for a total time of 7 months and 12 days, the duration of the experiment.

Since Bacot & Martin first demonstrated the vector capacity of individual fleas and Golov & Ioff revealed their role in the preservation of the disease from one year to another, there has developed a tendency to concentrate study on the individual flea rather than to work with fleas *en masse* as was previously done. Though mechanical transmission by large numbers of fleas was known to be of epizootiological importance, biological transmission by the blocked flea was considered of greater importance epidemiologically.

Meyer (1938) has stated that the important distinction made by George & Webster (1934) between infective (pestigenous) fleas and infected (pestiferous) fleas has not been sufficiently emphasized. He further remarks that only occasionally does a flea which feeds upon an animal with a plague bacteraemia become blocked and capable of infecting, i.e. a biological transmitter. He also states that: '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.'

According to Eskey (1938) the individual feeding of fleas from the time of their infection until death will provide exact knowledge of the period required for blockage to occur and the length of time that the insects can survive infection. Also if different species of fleas possess different abilities to transmit, and if infection persists longer in some species than in others, the character of plague epizootics in all probability will vary according to the species of fleas involved. Over eighty species of fleas had been collected from wild rodents in the western United States by 1938, and new species are being discovered every year.

Eskey & Haas (1940) conducted transmission experiments with individual fleas collected from both wild rodents in areas known to be sylvatic plague foci, and from domestic rats. All fleas were infected on guinea-pigs with a terminal bacteraemia. The faeces of each flea was collected and inoculated into a guinea-pig to determine the presence of plague organisms. When fleas were proved to be infected they were fed thereafter on healthy guinea-pigs. Plague transmissions were secured with two species of rat fleas and thirteen species of wild-rodent fleas and 9 % of the fleas infected transmitted. Both sexes transmitted, but of the total females infected, 11 % transmitted, while less than 4 % of the males did so. Many of the infective fleas transmitted the disease to more than one animal. Multiple transmissions by single fleas were accomplished by twelve species. One species was as likely to transmit the infection several times as was another. One *X. cheopis* female infected ten guinea-pigs. One ground-squirrel flea, an *Opisocrostis labis* (Jordan & Rothschild, 1922). male, infected eleven animals. This was the largest number of transmissions by a single flea.

Wheeler & Douglas (1941) presented an experimental method by which the vector efficiency of a species could be given as a numerical value. The advantage of such a procedure is that it enables the worker to compare the vector efficiency of different flea species through the use of a standardized technique. Following is a brief description of this procedure. Three potentials of a species are considered: the infection potential, the vector potential and the transmission potential. The infection potential is based on the percentage of fleas which can be proved to be infected through demonstration of the presence of organisms in the faeces. The vector potential is determined by the percentage of fleas which may transmit plague to animals. The transmission potential is the average number of transmissions accomplished by the infective fleas when fed once daily on individual white mice. The product of these three factors represents the number of transmissions effected by a given number of fleas and is designated as the vector efficiency of the species. Stated more simply, the vector efficiency is the ratio of daily transmission to total number of fleas used in the experiment.

In a later paper, Wheeler & Douglas (1945) give a detailed report of results obtained with this

method in vector studies, where they used it chiefly to compare the transmission abilities of the rat flea *Xenopsylla cheopis* and the ground-squirrel flea *Diamanus montanus* (Baker, 1895). The latter flea was found in their experiments to be nearly twice as efficient a vector of plague as was *Xenopsylla cheopis*, which until that time had been considered the vector *par excellence*.

MATERIALS AND METHODS

Selection of fleas

For the investigation of plague vectors to be detailed in the paragraphs which follow, ten species of fleas were selected. *Xenopsylla cheopis* (Rothschild, 1903), and *Diamanus montanus* (Baker, 1895) were selected for study for two reasons. First, they were to be used as a gauge in the development of techniques; and secondly, it was desirable to determine if results comparable to those of other workers who had used these species could be obtained. *Pulex irritans* Linnaeus, 1758 was selected because of its wide host range and frequent abundance in domestic situations. *Oropsylla idahoensis* (Baker, 1904) which naturally parasitizes many different species of ground squirrels within possibly the greatest geographical area of any North American squirrel flea, was chosen because of its wide host range. Although previous studies had not incriminated *O. idahoensis* as a vector, it has been collected from prairie dogs, chipmunks, marmots, tree squirrels and skunks, according to information assembled by Jellison & Good (1942). *Nosopsyllus fasciatus* (Bosc., 1801) was studied because it usually is the most common flea of domestic rats in temperate zones, and is therefore of epidemiological significance in the cooler climates where rats and plague exist. *Echidnophaga gallinacea* (Westwood, 1875), which was found infected in nature by Wheeler, Douglas & Evans (1941), was chosen for that reason. This flea has probably the greatest number of hosts, both mammalian and avian, of any species of flea, and is distributed in subtropical and south temperate climates throughout the world. It is commonly found on domestic rats, ground squirrels and barnyard animals.

Orchopeas sexdentatus sexdentatus (Baker, 1904) was considered an important flea to include in the studies since the species is found everywhere on rodents of the genus *Neotoma* (wood rats). These rats, which are known to suffer from spontaneous plague, readily take up a domestic existence in rural areas, and are consequently of epidemiologic significance. *Opisodasys nesiotus* Augustson, 1941 is found primarily on western deer-mice (*Peromyscus* spp.) but also parasitizes other members of the Cricetidae as well as the Muridae. *Megabothris abantis* (Rothschild, 1905) has been taken from a variety of hosts in mountainous and northern climates and was selected because it was the only flea found on *Microtus longicaudus* in the vicinity of a ground-squirrel epizootic at Lake Tahoe, California, in 1942. The role of *Microtus* spp. as winter reservoirs of plague has been emphasized by Tickhomirova, Sagorskaja & Iljin (1935) and by Meyer (1946). *Malaraeus telchinum* (Rothschild, 1905) was considered important for study because it is frequently found to infest Cricetidae in numbers exceeding all other fleas. It is at times the most prevalent flea of *Microtus* spp. Wood rats also harbour this parasite, as do occasionally domestic rats.

Source of fleas for study

All fleas of the species *Xenopsylla cheopis* and *Nosopsyllus fasciatus* used in this study were taken from *Rattus norvegicus* found on one of the dump grounds of the City of San Francisco. *Opisodasys nesiotus* and *Malaraeus telchinum* were collected from field mice in Marin County, near San Francisco Bay. *Orchopeas sexdentatus sexdentatus* specimens were obtained from wood rats at the Hooper Foundation Field Station, Calaveras Dam, Alameda County. *Diamanus montanus* was taken from California ground squirrels at the station, and *Pulex irritans* was collected from deer beds in this same area. *Echidnophaga gallinacea* was found on *Rattus norvegicus* in Sutro Forest near the Hooper Foundation in San Francisco. *Megabothris abantis* was taken from *Microtus longicaudus* near Myers, California, at the south end of Lake Tahoe, while *Oropsylla idahoensis* was secured from *Citellus beldingi* in the same area.

Rearing the fleas

All species of fleas used in the study were reared in the laboratory with the exception of *Pulex irritans*. This species could not be reared in the laboratory on either rats or guinea-pigs, while other species did quite well under these conditions. *Oropsylla idahoensis* reproduced more abundantly on a ground squirrel than on a laboratory rat, and was kept in culture on the former species, usually *Citellus beecheyi*; *C. beldingi* and *C. townsendi* were also used. *Malaraeus telchinum*, *Megabothris abantis* and *Opisodasys nesiotus* were reared on *Microtus*, since they survived better on this host than on the laboratory rat. *Orchopeas sexdentatus sexdentatus* was reared on both laboratory rats and wood rats, and did well on both animals. *Nosopsyllis fasciatus*, *Xenopsylla cheopis* and *Echidnophaga gallinacea* were all reared on white laboratory rats.

The method of rearing fleas was similar to that employed by Douglas & Wheeler (1943). Fleas and their hosts were kept in 5 gal. earthenware crocks with a glazed surface. The top edges of the crocks were fitted with metal bands with flanged ends,

which were tightened by a bolt and nut. These bands extended slightly above the top of the crock and had holes bored on each side so that a metal rod could be slipped over the top of a heavy piece of metal screen, cut to fit the top of the crock and set inside the band. About 2 in. of wood shavings were placed in the bottom of the crock; one or two teaspoonfuls of dry, powdered horse or sheep blood was then added, along with the same quantity of dried, ground brewers' yeast, as recommended by Sikes (1931). The shavings were slightly moistened and the host and fleas were placed in the crock and kept in a room where the temperature was maintained at 70–75° F., with 80–90 % relative humidity.

The length of time that an animal may be kept in a crock depends upon the amount of urine it produces. When the shavings become too soggy with urine, the immature stages of fleas do not develop because of unfavourable physical conditions and the presence of an excessive amount of ammonia, which is produced by bacterial action on the urea. *Microtus* and wood rats are not offenders in this regard, as they urinate but little. *Citellus beecheyi* and laboratory rats, on the other hand, produce urine in considerable quantities. It was found that if the wood shavings in the bottom of the crock were treated with water from a jar which contained a quantity of copper shavings, and allowed nearly to dry before use, the quantity of copper ions deposited on the shavings was sufficient to poison the bacteria-produced urease for some weeks; the host animal could consequently be kept in such a crock for a longer time. Confined host animals were not given drinking water but were supplied with fresh carrots, sunflower seeds, and rolled oats three times a week. The laboratory rats received, in addition, dog chow.

Maintenance of pure cultures of fleas

Animals were brought in from the field and placed in crocks with their own ectoparasitic fauna intact, at the start of a culture. Ectoparasites collected from many other animals of the same species which inhabited the area were sometimes placed in the crock with the host animal. After several weeks had passed and fleas had begun to breed in numbers, a large sample of fleas was taken out of the crock, mounted and identified. By a careful study of the morphology of cleared and mounted specimens, characteristics were discerned which could be used to separate uncleared living specimens according to species. Males are usually easy to separate; females are more difficult, but of the species used in this study, all can be readily separated while alive. To facilitate an examination of the living flea, a large cover-slip was fastened near one end of a glass slide with a piece of cellulose tape, leaving the other end of the cover-slip free. A flea can readily be placed under this with the aid of a hand bulb aspirator, and the cover-slip easily held in place with the index finger while the insect is being examined. After living fleas had been separated to species, pure cultures were maintained. Periodically, a large sample of fleas was removed from each stock culture and examined, both by clearing and mounting for identification and by examination of living specimens. It was felt that this safeguard was sufficient to assure the maintenance of pure culture. All fleas used in experiments were removed from the crock with the aid of a suction pump.

Feeding infected fleas

Mass transmission experiments were done in earthenware crocks. Various hosts were used in these experiments, dependent upon the species of fleas being studied. Five-gallon crocks were used for all experiments except those employing *Pulex irritans*, when a crock 18–20 in. deep was used. A note of caution with regard to the jumping ability of these different species of fleas might well be interjected here. A 5 gal. earthenware crock with inside dimensions of slightly over 12 in. in height and 11 in. in diameter will not confine *P. irritans*. Specimens of this species were seen on several occasions to jump out of a 12 gal. earthenware crock which had been filled to within 13 in. of the top with dirt. Martini (1923) states that this species can spring 32 cm. *Echidnophaga gallinacea*, while a good jumper, does not approach the proficiency of *Pulex irritans*. Most rodent fleas studied were poor jumpers and could be easily retained in a small enamelware pan about 4 in. high.

Fleas used in individual feeding experiments were kept in small screw-cap shell vials. The top of the metal screw-cap was removed and a piece of no. 72 grit gauze pasted in. This material readily retains even the males of small species of rodent fleas. Between feedings the vials were stored over a saturated solution of sodium carbonate at a temperature of 70° F., in sealed refrigerator pans. This supplied a relative humidity of 92 %.

Individual flea feeding was done on white mice. All mice used in feeding experiments were housed in individual cages of the type devised by Douglas & Wheeler (1941). The susceptibility of the mouse strain employed in the studies has been determined and is given in Table 1. A new mouse was used daily to feed each individual flea in the first experiment with a species. In later experiments a new mouse was not used daily for the first 3 or 4 days, as fleas rarely block in less than 4 or 5 days.

A combination mouse-holder and feeding tube simplified the technique of feeding individual fleas. A rectangular piece of $\frac{1}{4}$ in. mesh fine gauge hardware cloth $4 \times 4\frac{1}{2}$ in. in size was cut and rolled over

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in a circle on its long axis so that a piece slightly less than ½ in. long remained out of the roll. The edge of this piece was then turned down slightly so that it made a firm support for the feeding tube and prevented its turning over. A small piece was cut out at the top of the roll, slightly nearer one end, to expose the shaved abdomen of the mouse. Next a piece of wire was soldered to the holder in such a position that a loop in the wire would hold a flea vial tightly against the abdomen of the mouse. By employing these devices many fleas could be fed simultaneously and attention did not have to be concentrated on the feeding of each individual flea (see Plates 4, 5).

Table 1. *Mouse susceptibility test for* Pasteurella pestis

Days after inoculation	No. of mice that died		
	Group I	Group II	Group III
1	0	0	0
2	0	0	129
3	7	1	395
4	4	9	173
5	2	10	115
6	0	6	66
7	3	5	43
8	0	6	23
9	4	4	15
10	7	3	24
Survivors	20	17	44
Survivors (%)	42	27	4
Total	48	62	1064

Group I received 4–8 organisms subcutaneously from a 24 hr. broth culture of the 'Shasta' strain.

Group II received 10–20 organisms subcutaneously from a 24 hr. broth culture of strain '172', freshly isolated from a ground squirrel.

Group III received 10–20 thousand organisms subcutaneously from a 24 hr. broth culture of the 'Shasta' strain.

It is a simple matter to place a mouse in this holding device. After clipping the hair from the abdomen with electric clippers, the mouse is held by the tail in front of the holding tube. It will usually enter the tube readily; the tube ends, when plugged with cotton, secure the mouse firmly in place. After a number of mice have been placed in holding tubes the tops of the shell vials, which contain fleas, are removed, and the mouse holding tube inverted over the open vials, the latter fitted tightly against the smooth skin. This completed, the holding tubes are then placed in an enamelware pan.

Twenty to thirty fleas are fed simultaneously. During feeding, seven mice will conveniently fit in a refrigerator pan. If the pans are covered, the resulting darkness will facilitate feeding. Fleas allowed to remain in this position for 15–30 min. are given ample opportunity to feed and a great saving in time is made.

The easiest method of removing a flea from the abdomen of a mouse is to turn all the holders on their sides for a few minutes, permitting specimens to hop down into the vials. Those which do not voluntarily descend will frequently fall down if the holding tube is inverted and the vial flipped with the fingers a few times. Some species hold on more tenaciously than others, and a few specimens, particularly those which have refused to feed, will nearly always do so. These can be removed from the mouse with a suction pump, or with small forceps if care is taken not to injure them.

After a flea had been given an opportunity to feed on a mouse, it was examined to determine whether it had fed, refused to feed, or was blocked. Lightly pigmented fleas, when placed under a coverslip and held up to the light, show red ventriculi if they have fed. Dark-pigmented fleas must be examined microscopically. All fleas which did not have bright red, engorged ventriculi were also examined microscopically. Blocked fleas which have aspirated blood show bright red blood in the oesophagi but not in the ventriculi. Those partially blocked, in which the oesophagi or proventriculi are not occluded, show blood in both the ventriculi and the oesophagi. This condition, which Bacot (1915) has described, is caused by the growth of organisms around the proventricular valves, resulting in their disfunction. In such a flea, the blood runs freely from the oesophagus to the stomach and back again. Fleas which had blocked, but which did not aspirate blood so that the oesophagi were red just anterior to the proventriculus were not definitely classified as blocked. Consequently, some specimens were no doubt blocked at times when they were not so recorded. No flea which died before the first three feedings were completed was included in the data for its species.

Infecting the fleas

Bacot & Martin (1914) found that the white mouse was a much more satisfactory animal to use in infecting fleas with plague organisms than was the white rat, since the former developed a more severe septicaemia upon becoming infected. The desirability of using the white mouse was further emphasized by Douglas & Wheeler (1943). The Hooper Foundation 'Shasta' strain of *Pasteurella pestis* was used throughout these studies. This has proved to be a stable strain of constant high virulence. An inoculum of 0·1 c.c. of a 10^{-3} dilution of 24 hr. beef heart-hormone-broth, which contains ten to twenty thousand organisms, produces a demonstrable septicaemia in white mice in 2–3 days

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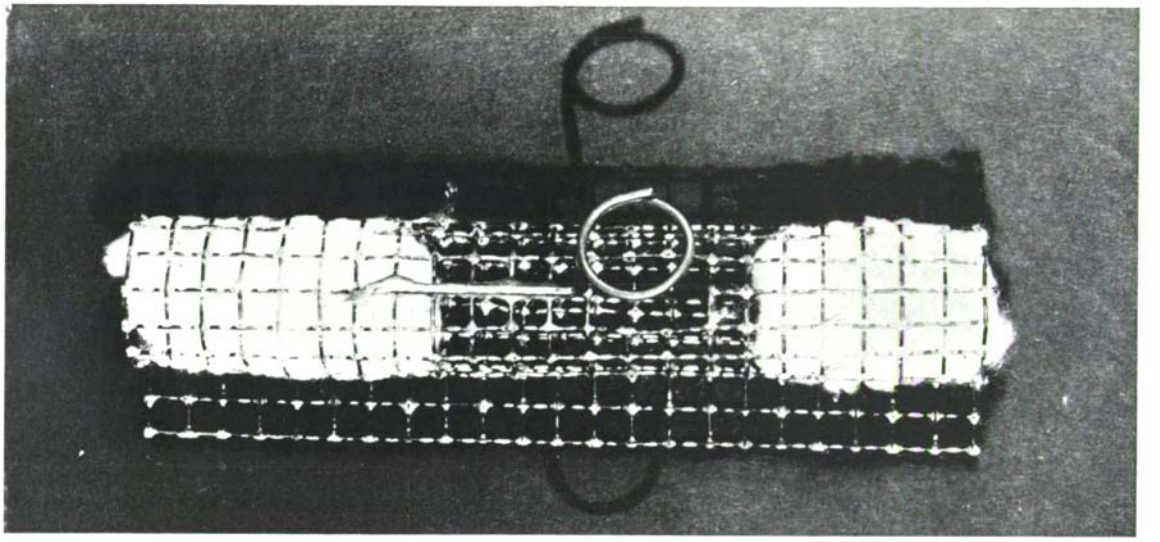

Fig. 1. Mouse holder and feeding tube from above.

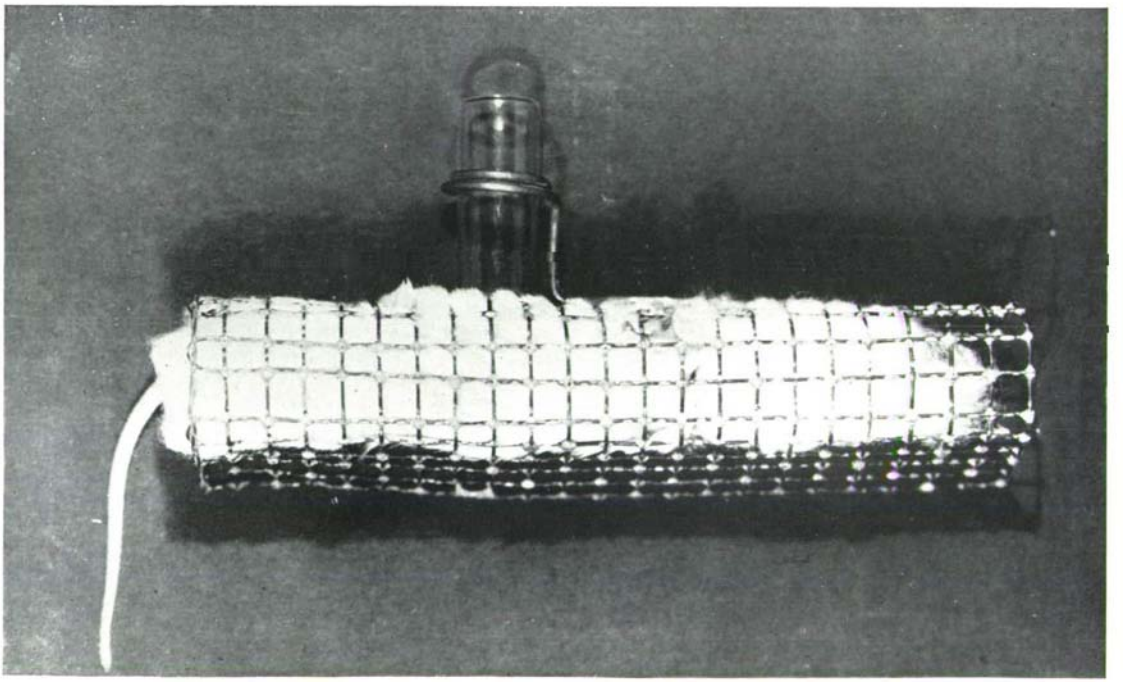

Fig. 2. Mouse holder and feeding tube with mouse and flea vial, side view.

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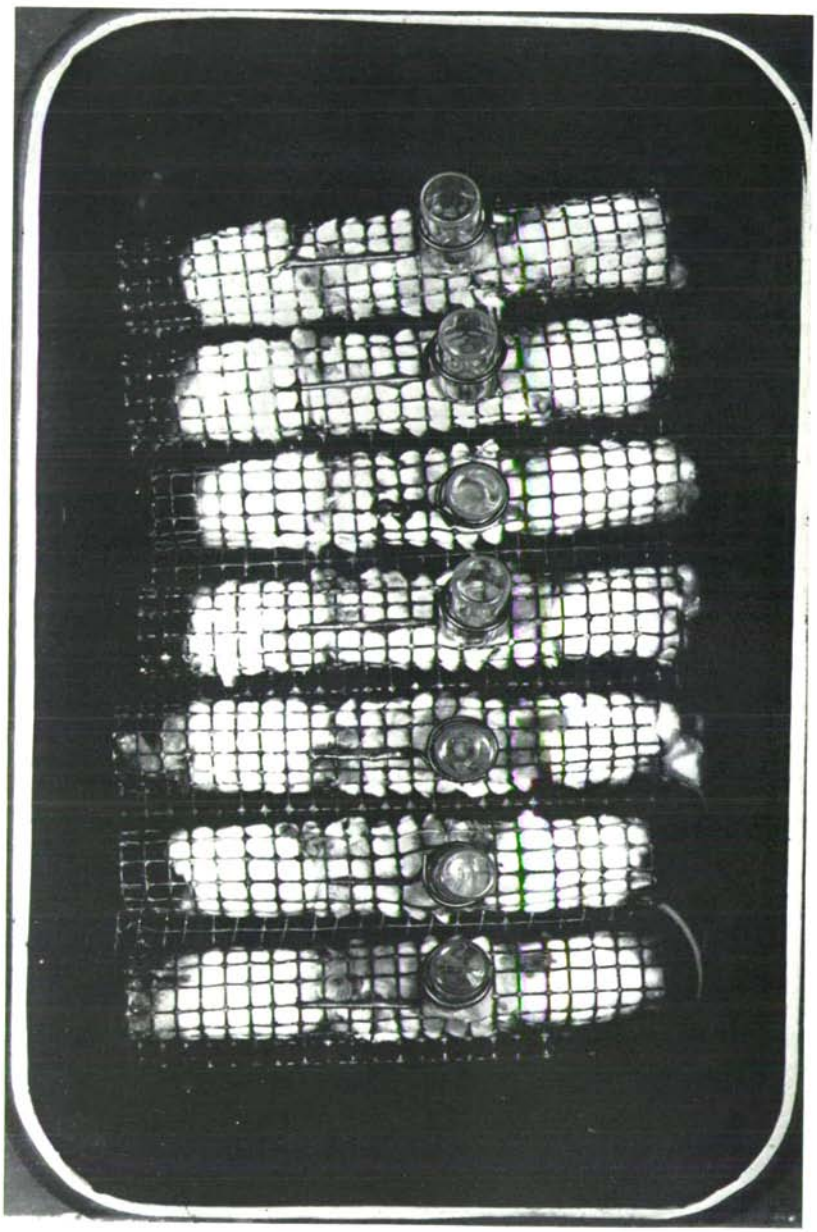

Fig. 3. Enamel-ware pan containing seven holders and feeding tubes with mice and flea vials.

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when introduced by either the subcutaneous or intraperitoneal route. The septicaemia is most intense just before death, and it is at this time that the fleas are fed. Before fleas were infected on a mouse, a thin smear of tail blood was examined microscopically to determine the degree of bacteraemia. In no case was a mouse used for infecting fleas unless many organisms were evident in every field. There were often twenty-five to forty, and sometimes fifty to seventy organisms per field. The degree of bacteraemia frequently becomes so intense just prior to death that there is one or more organisms for every red-blood cell. Mice often died within 5 min. or less after fleas had been placed on them, but a high percentage of fleas were, nevertheless, found to have fed in this short interval. As a preliminary experiment some fleas were ground and inoculated into mice and others were ground and plated immediately after an infecting blood meal. It was found that a flea could not feed on mice with a terminal bacteraemia without ingesting organisms. Consequently, all fleas which were thereafter observed to have fed on such mice were considered infected.

Eskey & Haas (1940) infected fleas on guinea-pigs. To determine with certainty that the fleas were infected, they were fed repeatedly on infected guinea-pigs, until organisms became demonstrable in the flea faeces by producing plague when inoculated into guinea-pigs. Douglas & Wheeler (1943) showed that the faeces of infected fleas do not always harbour organisms. In fact, they found that only 25 % of infected *Xenopsylla cheopis* and 65 % of infected *Diamanus montanus* excreted viable organisms. In the present work, dead fleas were frequently proved to be infected upon their inoculation in mice, even though organisms could not be cultured from the faeces during life. The procedure of culturing faeces was soon discarded for this reason and thereafter fleas were ground and inoculated at the time of death or at the termination of experimental feedings. Upon the death of an experimental animal which had been used either in feeding tests or in the inoculation of a dead flea, a sample of its heart blood or a spleen impression was cultured to attempt recovery of organisms. A positive report in experimental data indicates isolation of *Pasteurella pestis* from the animal.

Echidnophaga gallinacea

E. gallinacea presented a difficult problem because the adult insect is a permanent parasite and could not be fed periodically on animals as were the other fleas studied. It attaches itself by preference around the muzzle, the ears or some other location on the head. Mice clean such fleas off rapidly, devouring many of them. Infection was accomplished using rats to obtain infected fleas for both mass and individual transmission experiments. Rats were inoculated with *Pasteurella pestis* and, when obviously ill, had large numbers of *Echidnophaga gallinacea* placed on them. Most of the fleas quickly made their way to the rat's head and attached. As it is not known when these fleas begin to feed, nor how long they feed at any one time upon attaching, it could not be determined with certainty if they fed at the time of intense bacteraemia.

Individual transmission experiments encountered many obstacles. Different feeding devices were tried. Small wooden capsules with grit gauze bottoms and tops were sewed to the ears of guinea-pigs and a single infected flea put into each capsule. Sterile abscesses frequently developed and the capsules fell off. Finally, the gelatin capsule method of feeding was used. Following is a description of the technique: no. 5 hard-gelatin capsules are used. The end of the inside part of a capsule is cut away smoothly with a razor-blade and the resulting cylinder is stuck to the top surface of a mouse's ear with collodion, taking care that no collodion reaches the inside of the capsule. A flea is put in this cylinder with the aid of a bulb aspirator, and the top of the capsule quickly put in place. The chief objection to this method is that the capsule will fall off in about a week, when growth of hair detaches the collodion. However, mice removed very few fleas from their ears after the first several days' attachment had elapsed.

EXPERIMENTAL

Xenopsylla cheopis

In order to determine the biological transmission capability of *X. cheopis*, fifty-three fleas were infected and fed individually thereafter. The data on these fleas are given in Tables 2, 3 and 4. Approximately 20 % of the fleas that did not block lived for over a month. The failure of captive fleas to feed regularly was considered the chief factor contributing to their early death. This species may feed daily for a short time and then refuse to feed for one or more days. The fifty-three fleas were given, among them, a total of 718 opportunities to feed. There were 357 refusals and 361 attempts to feed, not all of which were successful since many fleas were blocked at various times. Twenty of the fifty-three fleas became vectors and transmitted altogether a total of thirty-five times, for a ratio of 35/53 or 0·66. Seven transmissions were effected by five fleas which were able to feed at the time they transmitted, demonstrating the proventricular valve disorder described by Bacot (1915). Three of these fleas transmitted once each, two of them blocking later, the other apparently having cleared itself of organisms, as it proved negative upon subcutaneous inoculation in a white mouse. Two of

the fleas in this same group of seven transmitted twice each and later blocked. There were twenty-nine attempted feedings at which fleas were *X. cheopis*, in their experiments, proved unable to infect rats at the first blood meal after a 15-day fast, but became infective less than 12 hr. later.

Table 2. Xenopsylla cheopis. *Blockage and transmission data by individual fleas*

	Days after infectious meal																					
Flea	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	S.Cut.
1	.	.	.	.	.	.	BT	N	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
2	.	.	.	.	.	.	.	.	.	.	BT	N	D	.	.	.	.	.	.	.	.	.	*p*
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B	D	*p*
4	.	.	.	.	.	.	.	.	BT	D	.	.	.	.	.	.	.	.	.	.	.	.	*p*
5	.	.	.	.	.	.	.	.	.	.	BT	N	D	.	.	.	.	.	.	.	.	.	*p*
6	.	.	.	.	.	.	.	.	.	.	.	.	.	B	D	.	.	.	.	.	.	.	*p*
7	.	.	.	.	.	.	.	BT	B	BT	BT	BT	D	.	.	.	.	.	.	.	.	.	*p*
8	.	.	.	.	RT	.	.	.	.	.	.	.	.	.	.	.	.	.	B	BT	D	.	*p*
9	.	.	.	.	.	.	.	.	.	.	BT	B	D	.	.	.	.	.	.	.	.	.	*p*
10	.	.	.	.	.	.	.	.	BT	BT	D	.	.	.	.	.	.	.	.	.	.	.	*p*
11	.	.	.	.	.	.	.	.	.	.	.	B	D	.	.	.	.	.	.	.	.	.	*p*
12	.	.	.	.	.	N	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
13	.	.	N	N	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	—
14	.	.	.	.	.	.	.	.	.	N	N	N	N	N	D	.	.	.	.	.	.	.	—
15	.	.	.	.	.	.	.	.	N	N	N	D	.	.	.	.	.	.	.	.	.	.	—
16	.	.	.	.	.	N	N	N	N	D	.	.	.	.	.	.	.	.	.	.	.	.	—
17	.	.	.	.	.	.	.	N	N	D	.	.	.	.	.	.	.	.	.	.	.	.	*p*

Feeding started 1 day after the infectious meal. The fleas were 3 days old when infected and had a blood meal 2 days before the infectious meal. R = fed; B = blocked; T = transmitted plague; N = refused to feed; D = flea died; S.Cut. = flea triturated and inoculated subcutaneously upon its death; *p* = mouse died of plague; — = survived inoculation.

Table 3. Xenopsylla cheopis. *Blockage and transmission data by individual fleas*

	Days after infectious meal																			
Flea	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	26	27	S.Cut.
18	.	.	.	.	.	.	.	.	.	BT	B	N	N	D	.	.	.	.	.	*p*
19	.	.	.	.	N	N	N	N	N	BT	BT	D	.	.	.	.	.	.	.	*p*
20	.	.	.	.	N	N	N	N	N	N	B	D	.	.	.	.	.	.	.	*p*
21	.	.	.	.	RT	R	N	BT	N	BT	D	.	.	.	.	.	.	.	.	*p*
22	B	N	N	N	N	B	D	.	.	.	.	.	.	.	.	.	.	.	.	*p*
23	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	B	B	D	*p*
24	.	.	.	.	.	.	.	B	N	BT	D	.	.	.	.	.	.	.	.	*p*
25	.	.	.	.	.	.	.	.	.	B	B	D	.	.	.	.	.	.	.	*p*
26	.	.	.	.	.	.	.	.	.	B	D	.	.	.	.	.	.	.	.	*p*
27	.	.	.	.	.	.	.	.	.	BT	N	D	.	.	.	.	.	.	.	*p*
28	.	.	.	.	B	N	N	N	N	N	D	.	.	.	.	.	.	.	.	*p*
29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	D	.	*p*
30	.	.	.	.	.	B	N	B	N	N	N	D	.	.	.	.	.	.	.	*p*
31	.	.	.	.	B	N	N	N	N	BT	D	.	.	.	.	.	.	.	.	*p*
32	.	B	N	N	N	N	N	N	N	N	D	.	.	.	.	.	.	.	.	*p*
33	.	.	.	.	.	.	.	.	.	B	B	D	.	.	.	.	.	.	.	*p*
34	.	.	.	.	.	.	.	.	N	N	N	D	.	.	.	.	.	.	.	*p*
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	D	.	.	.	*p*

Feeding started 2 days after infectious meal. Fleas 1 day old when infected. R = fed; B = blocked; T = transmitted plague; N = never fed; D = flea died; S.Cut. = flea triturated and inoculated subcutaneously upon its death; *p* = mouse died of plague; — = mouse survived inoculation of triturated flea.

obviously blocked though transmission did not occur.

Blanc & Baltazard (1942) stated that blockage probably depends on the multiplication of bacilli in fresh blood taken up at frequent feedings. The strain of *X. cheopis* used in the present studies did not exhibit the same characteristics as the fleas used by these French workers. In one experiment, fifty-one infected *X. cheopis* held at 70° F. and 92% relative humidity were starved for 8 days, then fed

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individually. Two of these fleas were blocked. One of these blocked fleas and another flea which was able to secure a blood meal at the time, transmitted. The mice died in 8 and 4 days respectively. In another experiment a group of fleas was infected and left at toom temperature in a covered jar for 9 days. Twenty-one fleas were recovered from this lot and fed. Three were blocked at the first feeding but were able to break their blocks on subsequent feeding.

Again, 214 infected fleas were divided among ten pill-boxes and held for 5 days, without feeding, at 70° F. with a relative humidity of 92%. They were then allowed to feed through no. 72 grit gauze on the shaved abdomens of white rats. One hundred organisms regurgitated will vary considerably in fleas of the same species as well as among those of different species, and even in the same flea at different times. This should be obvious from an understanding of the mechanism of a biological transmission of *Pasteurella pestis*. The following technique was devised to make possible the counting of regurgitated organisms: a mouse was placed on its back and taped to a board, its abdomen shaved, washed with soap and water, rinsed with saline and dried with sterile cotton. A blocked flea was then given an opportunity to feed on the abdomen of the mouse. After a few minutes the flea was examined microscopically to determine if fresh blood was present in the oesophagus, indi-

Table 4. Xenopsylla cheopis. *Blockage and transmission data by individual fleas*

	Days after infectious meal																	
Flea	8	9	10	11	12	13	14	15	16	17	18	19	20	21	30	31	39	S.Cut.
36	.	.	.	.	.	.	.	.	.	B	BT	N	B	D	.	.	.	*p*
37	.	.	.	B	B	N	BT	BT	BT	D	.	.	.	.	.	.	.	*p*
38	.	.	.	.	B	N	BT	BT	B	D	.	.	.	.	.	.	.	*p*
39	.	.	.	.	.	.	BT	RT	RT	.	.	.	.	.	B	BD	.	*p*
40	RT	.	.	.	.	.	RT	.	.	B	B	N	BT	D	.	.	.	*p*
41	.	.	.	.	.	.	RT	.	.	.	.	.	.	.	.	.	K	—
42	N	N	N	N	D	.	.	.	.	.	.	.	.	.	.	.	.	*p*
43	.	.	.	N	N	N	D	.	.	.	.	.	.	.	.	.	.	*p*
44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
48	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
49	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—
53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	K	—

Feeding started 3 days after infectious meal. Fleas of unknown age, but none over 14 days old. R = fed; B = blocked; T = transmitted; N = refused to feed; D = flea died; S.Cut. = flea ground and inoculated subcutaneously upon its death; *p* = mouse died of plague; — = mouse survived inoculation; K = flea killed.

and forty did not feed, thirty-six secured a blood meal, and thirty-eight were observed to be blocked. Of the 214 fleas, only seventy-four, or one-third, attempted to feed through the grit gauze. Of the fleas which did attempt to feed more than one-half were blocked.

X. cheopis, in these experiments, proved to be the vector *par excellence* that it has long been claimed to be.

Determination of the number of organisms present in the regurgitant of a blocked flea

No experimental results estimating the number of organisms present in the regurgitant of a blocked flea have been published. In the present investigation of this problem the flea *Xenopsylla cheopis* was used. It is to be expected that the number of

cating that regurgitation might have occurred. If it had, the mouse was etherized, the skin biopsied around the flea bite, and the area then covered with collodion. Most mice survived the effects of the operation. The biopsy material was ground in a mortar with sterile sand or carborundum and a 2 c.c. quantity of 1% peptone water was added. Grinding was continued until the skin was in finely divided particles. This suspension was plated in 0·2 and 0·3 c.c. quantities on beef-heart-hormone blood agar. In twenty-four trials no colonies grew on the plates; but in one attempt to recover organisms, thirteen colonies of *P. pestis* resulted from the plating of 0·3 c.c., and nine colonies grew from plating 0·2 c.c. of the original 2 c.c. biopsy suspension. This flea, therefore, had apparently regurgitated a minimum of eighty-eight organisms. That

this number was much too low will be shown later.

To check the technique, a series of biopsies was performed following inoculation of a mouse with material containing a known number of organisms. The original inoculum containing approximately 200 million organisms per c.c. (viable count) was diluted 1:10, 1:100, 1:1000, 1:10,000. Mice were inoculated intradermally with 0·05 c.c. of each dilution. These inocula contained approximately 1,000,000, 100,000, 10,000 and 1000 organisms respectively. Immediately after each mouse had been inoculated the skin around the area inoculated was biopsied to assure recovery of all inoculum. The skin was ground, diluted with 2 c.c. of peptone water, and plated.

The biopsy from the 1:10 inoculum should have produced 500,000 organisms per c.c. Counts of 550 and 570 colonies were obtained from 0·3 c.c. quantities of plated skin suspension, or an average of 1867 organisms per c.c. Thus less than 1 in 267 organisms was recovered. The 1:100 dilution biopsy yielded counts of thirty-four and forty from 0·2 c.c. platings of the original 2 c.c. biopsy suspension. This is an average of 185 colonies per c.c., when 50,000 organisms per c.c. were actually present in the inoculum. Less than one organism in 270 was recovered. The 1:1000 dilution biopsy should have contained 5000 organisms per c.c. Colony counts of seven and eight were obtained from 0·2 c.c. of the original 2 c.c. suspension. This is an average of thirty-seven organisms per c.c., or a recovery of one organism in 135. No organisms could be recovered from plates which had been sown with 0·3 c.c. quantities of a 2 c.c. suspension of biopsy material inoculated with 0·05 c.c. 1:10,000 dilution, which should have contained 500 organisms per c.c. It is evident that, in this series of experiments, no organisms could be recovered in the controls when less than 10,000 bacteria had been inoculated intradermally. The minimum experimental error was 135; that is, a recovery of one organism in 135. Using the foregoing figure to estimate the number of organisms transmitted by the flea in the experiment in which organisms were recovered, it is apparent that as many as 11,880 organisms (135×88) could have been present in the regurgitant. If the other experimental errors obtained are applied, the number would be 23,496 (267×88), or 23,760 (270×88).

The biopsied mice sometimes died of plague even when the biopsy was performed 10–15 min. after the flea had been first applied to the mouse. The longer the fleas were allowed to feed before biopsies were taken the higher was the percentage of mice that died of plague, as might be expected. The fact that mice died, even though the skin around the bite wound was excised shortly after the flea had bitten, would seem to indicate that organisms are deposited directly into the capillaries and that the primary stage of infection from a flea bite is frequently bacteraemia. This is not necessarily the case, however, since many biopsied mice did not die of plague. However, it has been shown that not all blocked fleas transmit plague; therefore, it cannot be determined if organisms were actually introduced into the mice each time a flea bit.

These experiments indicate that *P. pestis* cannot be recovered from the skin unless organisms are present in large numbers, and that even then only a small proportion can be recovered by the technique used. The method was revised in an attempt to recover a higher percentage of inoculated organisms. In the revised technique, each blocked flea was fed on a mouse's ear. After a flea had fed, the ear was cut off with hot scissors to prevent bleeding, sealed in a test tube, and frozen in an alcohol-dry ice bath. The hard, frozen ear was then ground, diluted and plated. It was thought that freezing the ear would make grinding easier; this proved to be so. However, in six trials with blocked fleas no organisms were recovered by this method. Experiments employing known numbers of organisms in measured amounts of inoculum were then made to check the technique. Two mouse ears were inoculated with 0·02 c.c. quantities (21,000 organisms) of a 1:100 dilution of a 24 hr. beef-heart-hormone broth culture of *P. pestis*, which gave a plate count of 105 million organisms per c.c. of the original culture. After following the above procedure, plates which should have produced 210 colonies each after the streaking of 0·1 c.c. biopsy suspension diluted 1:50 (2100 organisms per c.c.) produced 0, 0, 1 and 0 colonies. Controls run to determine if freezing had injured the organisms produced an average of seventy-seven of a calculated 210 colonies.

In a preliminary study to determine what the principle might be which prevented the recovery of organisms injected into mouse skin, three mouse ears and different amounts of plague inocula were ground with carborundum. A broth culture containing 126 million organisms per c.c. (plate count) was added in 0·1 c.c. quantities in the dilutions 1:100 (126,000 organisms), 1:1000 (12,600 organisms), and 1:10,000 (1260 organisms), one dilution to each ear trituration. Each ground ear was then diluted to 2·0 c.c. and plated in 0·2 c.c. quantities. These quantities should have produced 12,600, 1260 and 126 colonies respectively from 0·1 c.c. amounts of 1:100, 1:1000 and 1:10,000 dilutions added. The plating of the triturated mouse ear with the 1:100 dilution of *P. pestis* produced colonies too numerous to count; that of the 1:1000 dilution, counts of 279 and 281 or an average of 280 organisms, which is a recovery of one organism in 4·5. The

ground mouse ear with the 1:10,000 dilution of organisms produced no plague colonies, although by calculation 126 should have been present.

Continuing this preliminary study, 0·1 c.c. quantities of 1:100, 1:1000 and 1:10,000 dilutions were added to suspensions of mouse ears which had been well ground with carborundum and diluted to 2·0 c.c. These were allowed to stand for 15 min., then plated in 0·2 c.c. quantities in duplicate. The 1:100 and 1:1000 dilutions produced colonies too heavy to count and the 1:10,000 dilution produced an average of 120 colonies per plate, or 600 colonies per c.c., a recovery of nearly all of the organisms.

From results of these studies it is evident that plague organisms inoculated into the skin and connective tissue of mice or triturated with this material cannot be recovered if numbers below a certain threshold are employed. When inoculated into the skin and connective tissue and triturated or merely added to the material and then triturated, this threshold number is roughly 10,000–12,000 organisms for a quantity of skin the size of an adult mouse ear. When the ear is ground and diluted to 2·0 c.c. before adding the plague suspension nearly all organisms can be detected when as few as 1260 are added.

Mass transmission with Xenopsylla cheopis

Susceptible laboratory rats, when exposed to the bites of 100 infected *X. cheopis*, died as rapidly as when they were inoculated with a culture of *Pasteurella pestis* which did not produce death from the toxin present in the dosage given. It was observed that though mass transmission experiments with *Xenopsylla cheopis* were nearly always positive, those employing wild-rodent fleas were frequently negative. Variation in the number of fleas used was not the sole affecting factor, since large numbers of infected wild-rodent fleas do not always transmit plague. It is obvious, from the ensuing short period of survival after introduction of the organisms, that mechanical transmission results when large numbers of *X. cheopis* are used. Rats die within 3–5 days after the introduction of approximately sixty to one hundred newly infected fleas. This brief time disallows transmission by blocked insects as the causal factor in the transference of organisms. Furthermore, such transmissions are mechanical rather than biological.

The following experiment was performed to study the mechanism of mechanical transmission. Eight young adult rats were placed in separate crocks. These eight rats were used in pairs under four different conditions: (1) the toe-nails were clipped off and collodion applied so the rats were unable to scratch but could still bite and lick themselves; (2) the toe-nails were clipped off another set, collodion applied, the hind-legs hobbled and a rectangular metal collar put around the neck—these rats could neither bite, lick nor scratch; (3) metal collars were placed on two rats and the toe-nails were left intact so the rats could scratch, but neither bite nor lick themselves; and (4) two rats served as controls, without collars and with toe-nails intact; these could bite, lick and scratch.

One hundred *X. cheopis* which had been infected 24 hr. previously on moribund white mice were placed on each of the rats. These fleas were left on the rats for 24 hr.; then the rats were dusted with pyrethrum powder and washed in a 2 % phenol bath to sterilize all flea faeces. They were then dried and again dusted with pyrethrum before being placed in new crocks.

Two days after removal of the fleas the two control rats succumbed, as did those with clipped toe-nails but no collars, and those having collars and intact toe-nails. The two rats with collars and clipped toe-nails died, one on the third and one on the fourth day following removal of infected fleas. From these incubation periods it appears that rats which were subjected only to flea bites, and which could not lick, bite or scratch themselves, received a smaller number of infecting organisms. However, the small number of experiments made does not justify too definite conclusions.

Diamanus montanus

Sixty-six fleas of this species were infected for individual feeding experiments. Fifty-one (thirty-four females and seventeen males) lived 1 week; twenty-eight (nineteen females and nine males) lived 2 weeks; nine (six females and three males) lived 3 weeks; and one, a female, lived 4 weeks. In 744 opportunities to feed there were 520 feedings and 224 refusals. Two fleas, both females, blocked —one in 7 days, and the other in 9 days after the infectious meal. Both died the day following their blockage without having transmitted. However, one flea, a female in which blockage was not microscopically demonstrable, transmitted 4 days following the infectious meal, and then died on the 5th day. No other flea either blocked or transmitted. A third of the fleas were demonstrated to have retained their infection to death. These fleas did not prove to be nearly as efficient vectors as the strain of *D. montanus* studied by Wheeler & Douglas (1945).

Nosopsyllus fasciatus

Forty-eight fleas were fed individually in this experiment. All were of known age when infected: twenty-six were 1 day old and twenty-one were 2 days old. Most of these fleas would not feed regularly when given daily opportunities, but usually fed when given an opportunity on every other day. Once a specimen had blocked, however, it was given daily opportunities to feed.

This species apparently feeds better than *Xenopsylla cheopis* under the conditions of individual feeding described. Frequent feedings exercised a noticeable effect on the life span. Over half of these fleas lived 3 weeks, or a week longer than most *X. cheopis* survived. Blocked specimens behaved much as did *X. cheopis*, living on an average of but 2–3 days after blocking. One flea blocked in 4 days, and another in 29 days, the shortest and longest recorded periods, respectively.

The forty-eight specimens were given the opportunity to feed 716 times; there were 596 attempts or feedings and 120 refusals to feed. A total of eleven fleas blocked during the experiment, of which five transmitted. One flea which had never blocked transmitted, and another transmitted twice before it blocked solidly. There were in all ten transmissions by these six fleas (all females), seven of which resulted from feedings by fleas unable to secure a blood meal and three from feedings by two fleas which were not blocked so that they could not feed. Blockage was observed seventeen times in the eleven fleas in which no transmissions were effected. Twenty fleas, or 42%, remained infected until death, the others having effectively cleared themselves of the infection. The ratio of transmissions to fleas is 10/48 or 0·208, proving *Nosopsyllus fasciatus* to be a good vector of plague.

Table 5. Nosopsyllus fasciatus. *Blockage and transmission data*

	Days after infectious meal																				
Flea	5	6	8	9	10	11	12	15	16	17	18	19	20	21	22	23	24	29	30	32	S.Cut.
1	.	.	.	.	.	.	.	.	B	B	BT	R	.	.	.	.	D	.	.	.	—
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	RT	D	.	.	.	*p*
3	.	.	.	.	.	.	.	.	B	B	B	D	.	.	.	.	.	.	.	.	*p*
4	B	R	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
5	.	.	.	.	.	.	.	.	B	R	.	.	.	.	.	.	.	.	.	D	*p*
6	.	.	.	.	.	.	.	.	.	.	.	.	B	BT	BT	BT	D	.	.	.	*p*
7	.	.	.	.	.	.	.	B	D	.	.	.	.	.	.	.	.	.	.	.	*p*
8	.	.	.	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
9	.	.	.	.	B	BT	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
10	.	.	.	.	.	.	.	.	.	.	.	.	.	B	B	D	.	.	.	.	*p*
11	.	.	.	.	.	.	.	.	.	.	RT	RT	B	BT	D	.	.	.	.	.	*p*
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BT	D	.	*p*

R = fed; B = blocked; T = transmitted; D = died; S.Cut. = flea ground and inoculated subcutaneously upon death; *p* = mouse died of plague; — = mouse survived inoculation.

Malaraeus telchinum

Mass transmission with this flea was reported by Burroughs (1944). This species rarely feeds more frequently than on alternate days under the conditions of feeding previously described, if confined at 70° F. and 92% relative humidity. Many fleas refused to feed as often as this. Consequently, daily feedings were rarely offered, feedings frequently taking place at 3-day and occasionally 5-day intervals.

Of 115 fleas studied individually, all were alive at the end of 1 week; 101 lived 2 weeks, sixty-six lived 3 weeks, forty lived 4 weeks, twenty-six lived 5 weeks, six lived 6 weeks, and one lived 3 months. There were 898 feedings and ninety-five refusals. Five transmissions resulted, each by a different flea; no transmitting flea blocked in a manner demonstrable microscopically at the time of feeding. One of these was recorded as possibly blocked the day before it transmitted. No fresh blood was seen in its oesophagus, but there was a dark mass anterior to the proventriculus. None of the other four were suspected of being blocked at the time they transmitted. Many fleas harboured plague bacilli at the time of death, as shown by subcutaneous inoculation in white mice.

Fifty-six fleas were studied in one experiment. These were in two series, 1–30 and 31–56. Series 1–30 was fed for the first time 4 days after the infectious meal, while series 31–56 was fed for the first time on the 5th day after being infected. Three fleas in series 1–30 transmitted plague, one at the first feeding 4 days after the infectious meal, one at the tenth feeding, 31 days after the infectious meal, and one at the twelfth feeding, 38 days thereafter. Another flea in this series of thirty was suspected of being blocked at the fifth and sixth feedings, 15 and 19 days respectively after the infectious meal, but did not transmit. There was a dark mass present in the oesophagus which may have been obstructive, but no fresh blood was seen anterior to this mass.

In series 31–56, first fed individually on the 5th day after the infectious meal, one was suspected of being blocked on the third feeding, 11 days after the infectious meal, and transmitted on the fourth

feeding, the 14th day after infection. Another of this series was infective at the eighth feeding, 27 days after the infectious meal. These were the only two transmissions in this group.

A group of nineteen fleas used in a later experiment, and fed for the first time on the 2nd day after infection, contained one flea which might possibly have been blocked on the fourth feeding, 8 days after infection. None in this group transmitted. In still another series of thirty-nine infected fleas there were no suspected blocked fleas, and none which transmitted.

It is evident that these fleas are very poor biological vectors of plague, at least under laboratory conditions.

Table 6. Orchopeas sexdentatus sexdentatus. *Feeding record by days after the infectious meal of all specimens which blocked*

	Days after infectious meal																				
Sex	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
F	R	R	B	B	B	B	BD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
F	R	R	R	N	R	O	B	R	BT	B	D	.	.	.	.	.	.	.	.	.	.	.
F	R	R	R	R	B	B	BD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
F	R	R	R	N	B	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.
F	R	R	R	R	B	R	N	N	B	BT	BD	.	.	.	.	.	.	.	.	.	.	.
F	R	R	N	R	B	B	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.
F	R	R	R	R	B	B	B	B	B	B	B	D	.	.	.	.	.	.	.	.	.	.
F	R	R	B	R	R	R	R	N	R	N	R	R	O	N	R	R	R	R	N	O	D	.
F	O	R	O	R	O	R	O	R	O	R	O	B	B	B	BT	D	.	.	.	.	.	.
F	O	R	O	N	R	O	R	O	R	O	R	O	BT	BT	BT	D	.	.	.	.	.	.
F	O	R	O	R	O	N	R	O	R	O	R	O	R	O	N	B	N	B	B	N	B	D
M	R	R	R	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
M	R	N	R	R	B	B	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.
M	O	R	O	B	B	BT	BT	BT	D	.	.	.	.	.	.	.	.	.	.	.	.	.
M	O	R	O	N	R	O	R	O	R	O	B	D	.	.	.	.	.	.	.	.	.	.

F = female; M = male; R = fed; B = blocked; T = transmitted; D = died; O = no opportunity to feed; N = refused.

Orchopeas sexdentatus sexdentatus

In mass transmission experiments with this species, 100 fleas were placed on a white rat 24 hr. after their infectious meal. The rat succumbed to plague in 4 days; a new rat was put into the crock and it died in 6 days. In still another crock thirty-nine day-old infected fleas were placed on a rat, which died in 5 days. A second rat placed in the crock died in 3 days.

Thirty-one males and twenty-two females were used in individual transmission experiments. Fifty (twenty-nine males and twenty-one females) lived 1 week, thirty (eighteen males and twelve females) lived 2 weeks, and ten (seven males and three females) lived 3 weeks. The fifty-three fleas were given a total of 545 opportunities to feed. There were 466 feedings or attempts to feed, and seventy-nine refusals. It can be seen that this species fed better than did some of the others.

Fifteen fleas blocked, of which eleven were females and four were males. These fleas were blocked at fifty-three feeding attempts, but there were only nine transmissions, all accomplished by five fleas. Four females transmitted a total of six times: three once each, and one three times. One other flea, a male, transmitted three times.

The ratio of transmissions to specimens investigated is 9/53, or 0·17. The feeding data of all blocked *O. sexdentatus sexdentatus* is given in table 6. This experiment shows the species to be a capable vector, but inferior to some of the others investigated, under laboratory conditions.

Opisodasys nesiotus

This rodent flea was proved to be an efficient vector in mass transmission experiments with *Peromyscus maniculatus* used as host animals. Forty-six fleas were given a total of 505 opportunities to feed in individual transmission experiments. There were 344 feedings or attempts at feeding, and 161 refusals. Thirty-three fleas lived 1 week, nineteen lived 2 weeks, twelve lived 3 weeks, and two lived 4 weeks. Ten fleas were blocked at twenty-four attempted feedings.

Three transmissions were achieved by two of these blocked fleas. One flea was blocked on two consecutive days, transmitted each day, and died after attempting to feed on the 2nd day. The other flea blocked and transmitted on the 5th day after the infectious meal, then cleared and fed the next day, and lived fifteen more days without blocking or transmitting again. Another flea blocked in less than 41 hr., was blocked for three consecutive days and then died, never having transmitted during any of these attempts to feed. This is the shortest recorded period for blockage to develop in any species. Of the fleas 54% retained their infection

to death, the others having cleared their alimentary tracts of organisms. The three transmissions for this experiment give a ratio of 3/46 or 0·065. The particularly short extrinsic incubation period for some of these fleas may be an indication that the species might be a very good vector under more suitable conditions.

Echidnophaga gallinacea

Mass transmissions from rat to rat were repeatedly obtained with lots of twenty-five to one hundred fleas. Many fleas remain attached for several hours after the rat's death and apparently suck body fluids from the dead animal. Evidence of this was obtained when fleas taken from rats in advanced stages of decomposition produced heavy cultures of *Proteus* when triturated and plated on blood agar.

blocked, since all fleas with the exception of the one which transmitted twice were dead before or by the time the mice had died. The one flea which transmitted twice was dead by the time the second mouse it had infected succumbed. All fleas were dead at the end of 20 days and the experiment terminated.

Oropsylla idahoensis

A mass transmission experiment with twenty-nine infected *O. idahoensis*, using a young adult rat as the host, gave negative results and was terminated in 6 weeks. Another with thirty fleas also gave negative results and was discontinued in 5 weeks. A third mass transmission experiment gave positive results. In this experiment 110 fleas were used. Five weeks after infected fleas had been placed on it, the rat was anaesthetized, the fleas removed, and it was palpated to determine the

Table 7. Opisodasys nesiotus. *Blockage and transmission record*

	Days after infectious meal																							
Flea	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	S.Cut.
1	.	.	.	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
2	.	.	.	B	B	B	.	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
3	.	.	.	.	B	B	B	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
4	.	.	.	.	B	R	B	.	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
5	.	B	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
6	.	.	.	.	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
7	.	.	.	.	B	B	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*p*
8	.	.	.	.	BT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	*p*
9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BT	BT	D	.	.	.	.	*p*
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	BK	*p*

B = blocked; R = fed; D = flea died; T = flea transmitted plague; K = flea killed; S.Cut. = flea inoculated subcutaneously upon death; *p* = mouse died of plague.

It could not be determined if all fleas used in individual transmission experiments were infected, since there was no way to establish with certainty whether or not the living flea had fed during the period of intense bacteraemia. Eskey & Haas (1940) and Wheeler & Douglas (1945) have shown that faecal culture does not always reveal an infection in fleas. Therefore, to obtain an estimate of the number of fleas which had actually fed during the period of bacteraemia in the rat, twenty fleas of the lot which had been infected for individual transmission experiments were ground and plated on blood agar. Sixteen, or 80 % of these, proved to be infected. An additional forty-eight fleas removed from this same lot were placed in ear capsules on individual mice. Eleven fleas transmitted plague to the mice. One of these was still alive after the first transmission and was placed on a second mouse, which it also infected. Thus, twelve transmissions were effected by eleven of the forty-eight fleas, a ratio of 12/48 or 0·25.

The fleas which transmitted were probably

presence of buboes. Right axillary and inguinal buboes were palpable, so the rat was killed and dissected. *Pasteurella pestis* was recovered from these buboes.

None of the nine fleas recovered from the crock was found to be infected when inoculated into white mice. However, it was not definitely known whether these were the original fleas placed in the crock, for they might have been fleas which were bred there, since the experiment lasted 5 weeks. The fact remains, however, that no fleas were found infected at the termination of the experiment.

Sixty-one fleas were used in vector efficiency tests. Most of them lived 2 weeks, and many lived 3 weeks. At the end of a month only a few specimens remained alive, and the experiment was terminated. There were 451 feedings and 336 refusals. No flea blocked or transmitted. They fed irregularly, some specimens fasting for 5–7 days between meals. Digestion is probably slower in this species than in most of the others studied. The stomachs of these fleas were frequently one-half to

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two-thirds full of dark liquid material the day following feeding, and in approximately a fifth the blood was still red, indicating that very little digestion had occurred in the 24 hr. interval. The fleas quite efficiently cleared themselves of the organisms ingested in the infectious meal, for few were found to harbour the organisms at death.

Pulex irritans

Since *Pulex irritans* does not feed readily on mice, all the hair was clipped from the sick mouse to facilitate feeding. Two mass transmission experiments, one with sixty and one with eighty fleas, were made using young guinea-pigs as host animals. Fleas were placed on the guinea-pigs 24 hr. after taking an infectious meal, and these pigs died from plague in 15 and 10 days respectively. Rats were not used, as it had been previously determined that the species did not feed well on them. Jellison & Kohls (1936) have, however, reported that some strains of *P. irritans* are naturally adapted to rodents.

Fifty-seven specimens (twenty-nine males and twenty-eight females) were infected and used in individual transmission experiments. Fifty-three (twenty-five males and twenty-eight females) lived 1 week, thirty-three (sixteen males and seventeen females) lived 2 weeks, seventeen (eight males and nine females) lived 3 weeks, fifteen (seven males and eight females) lived 4 weeks, seven (one male and six females) lived 5 weeks, five females lived 6 weeks, and two females lived 7 weeks. Most specimens fed irregularly, rarely feeding each time when given daily opportunities. Most fed on alternate days, but some refused to feed as often as this. There were 421 feedings and 188 refusals. Of these fifty-seven fleas, one, a male, blocked in 11 days and then died the next day; it did not transmit, nor did any of the others.

Seven specimens were infected and starved for 9 days. At the end of this time they were given an opportunity to feed, and only four partook. None of the fleas blocked or transmitted during this experiment. Many of those used in individual feeding experiments were proved to be infected at the time of death upon inoculation into white mice. In fact, 31% of the fleas were found to be infected at death.

Megabothris abantis

In mass transmission experiments with this species, twenty-seven fleas which had been infected the previous day were placed on a *Microtus*. The animal succumbed to plague in 8 days. Another *Microtus* put in the same crock with the remaining fleas was infected and died on the fourth day after being placed there.

None of these fleas was of known age, but many were recently emerged imagos. Forty-eight infected fleas (nineteen females and twenty-nine males) used in individual transmission studies effected no transmissions. Twenty-eight males and eighteen females lived 1 week, twenty males and twenty-six females lived 2 weeks, four males and ten females lived 3 weeks, and one male lived 4 weeks. There were 437 recorded daily feedings and 63 refusals. One flea, a female, blocked on the 12th day after the infectious meal and died the following day without transmitting. One lot of fourteen fleas was not ground and inoculated subcutaneously at death, but was instead mounted and identified. Nineteen fleas, or 56% of those remaining, were infected at death.

Of twenty-seven other specimens infected and studied, fourteen were males, and thirteen were females. These fleas fed, among them a total of 306 times, and refused at sixty-nine daily feedings. Eight fleas (two males and six females) blocked and were so recorded at a total of twenty-one daily feeding attempts. The minimum extrinsic incubation period was 10 days, the maximum was 25 days and the average, 17·4 days. The minimum period of survival after first blocking was 1 day, the maximum 10 days, the mean 4 days. There were four transmissions, all accomplished by two fleas, both females, each transmitting twice. One flea transmitted while blocked, 22 days after the infectious meal, and was able on the following day to break its block and secure a blood meal, at which time it again transmitted. The other flea transmitted while blocked on the 25th and 26th days after the infectious meal. Eleven of these fleas (41%) were still infected at the time of death.

These fleas survived somewhat better than had others of the same species when used in the previous experiments. Fourteen males and twelve females lived 1 week, seven males and nine females lived 2 weeks, three males and six females lived 3 weeks, and two males and one female lived 4 weeks. The slightly increased survival time is not sufficient to account for the suddenly enhanced vector capacity of this species, however, nor to account for the additional number of fleas which blocked in this experiment as compared to the other experiment. White Swiss mice, which were used in this experiment, proved to be somewhat more resistant than the mice used in the other experiments. Therefore, although this data is not strictly comparable to the other, it is nearly so for all practical purposes. The ratio of all *Megabothris abantis* studied to transmissions obtained with the species is 4/75 or 0·053. The fact that this species was able to exhibit a capacity for transmitting, and that several fleas blocked in the latter experiment, while in the first experiments there were no transmissions and only one flea was observed to block, leaves some question as to whether the real vector efficiency has yet been obtained.

Table 8. *Feeding and transmission data of fleas studied*

	X. cheopis	*N. fasciatus*	*O. sexdentatus sexdentatus*	*O. nesiotus*	*M. abantis*	*D. montanus*	*M. telchinum*	*P. irritans*	*O. idahoensis*	*E. gallinacea*
No. studied	53	47	53	46	75*	66	115	57	61	48
No. feedings	718	596	466	344	743	520	898	421	451	48
No. refusals	357	120	79	161	132	224	95	188	336	0
No. blocked	31	11	15	10	9	2	0	1	0	11†
Total daily blocks	63	24	53	24	22	2	0	1	0	12
Percentage blocked	58	23	28	22	12	3	—	1·75	0	0·23†
No. transmissions by completely blocked fleas	28	7	9	3	3	0	0	0	0	12†
No. transmissions by fleas not completely blocked	7	3	0	0	1	1	5	0	0	0†
Total transmissions	35	10	9	3	4	1	5	0	0	12
No. infected at death	38	20	37	25	30*	17	31	18	4	‡
Percentage infected at death	72	42	70	54	40*	25·7	17	31	7	‡
No. that transmitted	20	6	5	2	2	1	5	0	0	11
Ratio transmissions to fleas used	0·660	0·213	0·170	0·065	0·053	0·015	0·043	0	0	0·250

* Only 61 inoculated at death.

† As these fleas could not be examined microscopically after each meal as were the others this data cannot be presented with certainty.

‡ Not inoculated at death.

DISCUSSION

Mass transmissions

It has been demonstrated that the ability of a particular species of flea to transmit *en masse* is not necessarily an accurate measure of its capacity to serve as a biological vector of plague. *Malaraeus telchinum* is quite capable of transmitting when it is placed in numbers on a susceptible host, but its ability to transmit plague biologically has been shown through individual transmission experiments to be very slight. *Oropsylla idahoensis* was also proved to be capable of transmitting *en masse*, but individual transmissions were not obtained in these studies, nor in those of Eskey & Haas (1940).

That normal fleas regurgitate stomach contents into the bite wound, as the mosquito is reported to do, has not been proved. This phenomenon is improbable, however, because of the valvular arrangement in the proventriculus of the flea. The bites of many fleas with soiled probosci may cause infection in a susceptible host animal, while the bite of one flea with a soiled proboscis would be unable to do so. The Indian Plague Commission, as previously mentioned, found that infection in animals *per os* is rare, and requires the ingestion of large numbers of organisms. The mice used in the present studies were very resistant to infection by mouth, a fact determined by placing large numbers of organisms in their mouths with a cotton swab. It was therefore concluded that the probability of a mouse becoming infected through ingestion of infected flea faeces was exceedingly slight. For a time all faecal droplets were cleaned off mice used in individual transmission experiments with a swab dipped in methyl alcohol; when this procedure was later discontinued no increase in the number of transmissions occurred. It is doubtful whether contaminated flea faeces on the unabraided skin can produce infection. The composition of the faecal pellet is such that it retains the organisms by its viscous consistency and they do not come into intimate contact with the skin. Furthermore, at the time of feeding, one of the most likely times for defecation to occur, the posterior of the flea is uppermost, and the faecal material is usually deposited on the animal's hair. Eskey & Haas, in thirty attempts, did not succeed in infecting guinea-pigs by rubbing the faeces of infected fleas into the scarified skin. In parallel experiments, portions of faecal material which had produced plague in guinea-pigs when inoculated subcutaneously gave negative results when rubbed into the scarified skin. They concluded that '. . . it may be doubted that the disease is ever contracted naturally through the agency of fecal deposits coming into contact with wounds or scratches'. Ioff (1941) takes exception to the conclusions of Eskey & Haas and cites the work of Bacot & Martin (1914) and Golov & Ioff (1925) in which different results were obtained. It appears that this mode of infection, though not impossible, is uncommon. Though, biologically, the individual flea is all-important in preserving plague in an enzootic state in areas where occasional sporadic cases of the disease occur in a sparse or highly resistant animal population, mechanical transmission by numbers of infected fleas is undoubtedly the more important means of disseminating the causative organism in a dense, susceptible population in epizootic times.

The mass transmission experiments with *Xenopsylla cheopis* in the present investigation might indicate that rats which were able to scratch and lick themselves received a larger infecting dose of organisms than did those which could neither scratch nor lick themselves and which were exposed only to the bites of many infected fleas. As all these rats died of primary septicaemic plague, the number of organisms acquired through licking is insignificant, since pneumonic lesions were absent. The bites of many fleas with contaminated probosci were, however, sufficient in all cases to introduce ample organisms to produce a fulminating infection. Perhaps one reason some fleas transmit more efficiently *en masse* than others is because they feed more frequently, and so bite a new host animal while viable plague organisms remain on the mouthparts.

Tumanski & Poliak (1931) list the various modes of plague transmission from rodent to rodent in the following order of importance: (1) crushing infected fleas with the teeth, infection occurring through the mucosa of the buccal cavity with subsequent involvement of the lymph nodes of the neck; (2) scratching and rubbing infected fleas and, rarely, their faecal droppings into superficial skin wounds or abrasions; (3) by the bite of fleas with plague-contaminated probosci following the sucking of blood from a diseased rodent; and (4) by the blocked flea.

Transmission in this order of importance is not supported by experimental evidence. It also is in contrast to the views expressed by Bytchov (1935). Furthermore, Simond, as early as 1898, showed that rats had to ingest large numbers of organisms before they became infected by the oral route. This applies not only to rats but to other rodents as well. The Indian Plague Commission discredited infection by ingestion of infected fleas in 1906. As for the second most important means of infection listed by Tumanski & Poliak, fleas are rarely crushed by a host animal, and in the laboratory both the Sciuridae and the Muridae are apparently oblivious to the insects until excessively parasitized. That method accorded third place, 'infection through bites of fleas with soiled probosci', is of major importance during epizootics where mass transmission is the rule rather than the exception. However, the bites of individual fleas with soiled probosci rarely provoke infection. The last method listed by these Russian workers is of primary importance in the persistence of enzootic plague and is most significant of all. Meyer (1941), on the basis of experimental evidence, emphasized the improbability of the first two methods listed by Tumanski & Poliak, and the unlikelihood of transference of infection mechanically by a single flea. An exact reversal of the order of importance as given by these Russian workers would be in agreement with the facts.

Individual transmissions

A knowledge of the vector efficiency of a species of flea acquired experimentally in the laboratory is probably, at best, only an approximation of its natural vector efficiency. This is inevitable because ignorance of the ecology of most species of fleas precludes the duplication of natural conditions. It is known that many underground rodent nests have relative humidities close to or at saturation. Knowledge of the prevailing temperatures, however, is limited. The effect of temperature variations on epidemic and epizootic plague has been studied, as previously disclosed, by the Indian Plague Commission, by Hirst, Brooks, Goyle and others. Eskey & Haas (1940) found that *Xenopsylla cheopis* became infective more rapidly when exposed to temperatures between 72 and 80° F. than when confined at a mean room temperature of 66° F. It is possible that more nearly optimum conditions can be created in the laboratory for some species, such as *X. cheopis*, and that the laboratory conditions set up are unfavourable for some rodent fleas such as *Megabothris abantis* or *Oropsylla idahoensis*, both cold-climate fleas. In their natural habitat these fleas may be much better vectors than the results of laboratory experiments would indicate. The importance of temperature relationships to these studies was emphasized by the Russian investigator, Dr I. Ioff, in a personal communication to Dr K. F. Meyer of 7 April 1946. Dr Ioff then stated that he believed future studies on the effectiveness of various vectors must give more careful consideration to external ecological factors, mainly temperature.

In nature fleas have an opportunity, except in deserted burrows, to feed at will, and may frequently remain on the host for long intervals. A blocked flea may have a much better opportunity to transmit plague under these conditions. It was observed in the present experiments, as in previous work, that many blocked fleas never did transmit, and that others did not transmit the infection at each attempt to feed. This phenomenon has been reported by Eskey & Haas, as well as by Wheeler & Douglas, and others before them. Had these blocked fleas been on a host for longer periods of time than permitted at laboratory feedings, they might have transmitted more frequently.

Stewart (1940) has stressed the fact that although host specificity is not so absolute a factor as was believed some years ago, most fleas do have certain animals or groups of closely related animals which they parasitize chiefly. The fleas of field mice, for example, are rarely found on the Sciuridae, nor do fleas of the Sciuridae commonly parasitize the Cricetidae. What effect the use of the white mouse

in feeding fleas not normally parasitizing the Cricetidae or Muridae had on the results of these studies is indeterminable from present knowledge.

Estimation of vector efficiency

To estimate the expected number of mice killed (total number of daily transmissions) per flea use the formula

$$\bar{X} \pm K_\alpha \sqrt{\frac{S^2}{N}}, \qquad \text{(I)}$$

where $\bar{X}$ is the average number of transmission per flea for the entire number of fleas studied, N the total number of fleas used in the studies,

$$S^2 = \frac{\text{Sum of sq. of total transmissions by indiv. fleas}}{\text{Total number of fleas} - 1} - \frac{\text{Total number}}{\text{Total number} - 1}\bar{X}^2,$$

and K_α is defined by $\int_0^{K_\alpha} \frac{e^{-\frac{1}{2}T^2}}{\sqrt{(2\pi)}}\,dT = \alpha$,* α being the probability that the estimating interval covers the true value. For example:

α	K_α
50 %	0·67
90 %	1·64
95 %	1·96

Thus if $\bar{X} = 0{\cdot}66$, $S^2 = 1{\cdot}076$ and $N = 53$, and $\sqrt{(S^2/N)} = 0{\cdot}1425$, applying formula (I) the result is $0{\cdot}66 \pm 1{\cdot}64 \times 0{\cdot}1425 = 0{\cdot}660 \pm 0{\cdot}234$. Then there is a 90 % probability that the interval $0{\cdot}660 \pm 0{\cdot}234$ represents the true expected value. This is only true if fifty samples, or thereabouts, are taken.

Method of determining if the variation in the vector efficiencies obtained in two experiments is real or due to chance

To test the hypothesis that the ratios of transmissions to total fleas differ only because of chance in two transmission experiments with a single species of flea, the following formula is applied:

$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}\right)}} = T. \qquad \text{(II)}$$

T has an approximately normal distribution with mean 0 and standard error 1. Example: $X_1 = 0{\cdot}43$, $\bar{X}_2 = 0{\cdot}66$, $N_1 = 49$, $N_2 = 53$, $S_1^2 = 0{\cdot}582$, $S_2^2 = 1{\cdot}076$. Applying formula (II):

$$\frac{0{\cdot}43 - 0{\cdot}66}{\sqrt{\left(\frac{0{\cdot}582}{49} + \frac{1{\cdot}076}{53}\right)}} = T$$

$$= \frac{-0{\cdot}23}{\sqrt{0{\cdot}0322}} = -\frac{0{\cdot}23}{0{\cdot}179} = -1{\cdot}28 = T.$$

* This is found in texts on statistical methods in the Tables of Probabilities and Ordinates of the Normal Curve Corresponding to given Deviations.

Using an α of 95 % one rejects the hypothesis as false when $T > +1{\cdot}96$ or $< -1{\cdot}96$. Thus if the hypothesis is rejected as false when T is in either of the $2\frac{1}{2}$ % tails of the normal, this procedure will also result in rejection of the hypothesis as false when true about 5 % of the time. That is, at a significance level of 5 %, the hypothesis will be rejected if $T > +1{\cdot}96$ or if $T < -1{\cdot}96$. In the above example the hypothesis would hold as T falls within the $+1{\cdot}96$ and $-1{\cdot}96$ limits. The conclusion would therefore be that the difference between $\bar{X}_1$ and $\bar{X}_2$ was due only to chance.

Xenopsylla cheopis

The theory of Blanc & Baltazard (1942) that blockage probably depends on the multiplication of bacilli in fresh blood taken up at frequent feedings is not borne out by investigation. *X. cheopis* studied in the plague laboratory of the Hooper Foundation were observed to block more rapidly and in greater numbers after starving for several days than when fed daily.

In the thirty-one fleas which definitely blocked in this series of experiments, the shortest extrinsic incubation period was 5 days, the longest 25 days and the mean 12·6 days. The greatest number of fleas to block on any one day was eight; these blocked on the 14th day. The average survival time after having blocked was 4·4 days, with a minimum of 1 day and a maximum of 17 days. Thirty-eight fleas (72 %) were infected at death, and fifteen (28 %) had effectively cleared themselves of infection. These latter fleas are seen, by referring to Table 4, to have survived much longer than the fleas in which an infection became established. Each infective flea transmitted an average of 1·75 times. There was a minimum of one transmission and a maximum of four for the infective fleas.

Wheeler & Douglas, holding their fleas at approximately the same temperature (20–23° C.), obtained a minimum extrinsic incubation of 6 days, a maximum of 33 days, and an average of 16 days, for fourteen blocked fleas of this species. The average duration of infectivity was 4 days with a minimum of 1 day and a maximum of 18 days. The phrase 'duration of infectivity' is not satisfactory as fleas will occasionally clear after blocking, feed many more times, then block again and die. These fleas are not infective during much of the interim. The infective fleas in Wheeler & Douglas's experiments transmitted an average of 1·36 times, with a minimum of one transmission and a maximum of three.

Eskey & Haas, studying the effects of temperature on the extrinsic incubation time of plague, obtained an average of 21 days for thirteen fleas

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held at from 50 to 70° F. (mean 66° F.) and an average of 15 days for fifteen fleas kept in an incubator at 72, 77 and 80° F. The latter temperature variations are probably too widely divergent to consider these fleas together, but in comparing them with the thirteen fleas held at room temperature, the results are interesting. An increased temperature apparently reduces the time required for blocking to occur. The average survival time of *X. cheopis*, after infecting the first experimental animal, was 2·8 days, and each infective flea transmitted an average of 2·1 times. Eskey & Haas's data is not strictly comparable with the other two sets of data, as different techniques were applied. Some infective fleas were allowed to feed on several animals in a single day, and for this reason, transmissions are not always 'daily' transmissions in their work.

It is seen that the three sets of results compare very closely. Comparing the vector efficiencies obtained from the three different experiments, in the present studies there were thirty-five transmissions for fifty-three fleas, or a ratio of 0·66; Wheeler & Douglas obtained twenty-one transmissions in studies with forty-nine fleas, for a ratio of 0·43; Eskey & Haas, employing 140 fleas, obtained fifty-nine transmissions, for a ratio of 0·42. The latter figure is nearly identical with that of Wheeler & Douglas. In interpreting the figures, however, it must be remembered that Eskey & Haas's transmissions are not classified as daily transmissions. Unfortunately, the latter workers did not include enough of their records in the report studied to allow statistical analysis of the data. The squares of the individual transmissions, which are lacking, are needed for such analysis. However, the findings of Wheeler & Douglas have been so analysed and compared with the present studies.

To estimate the expected number of transmissions by *X. cheopis* in this work, $N = 53$, $\bar{X} = 0{\cdot}66$ or $(\frac{35}{53})$

$$\text{and } S^2 = \frac{\text{Sum of the squares}}{N-1} - \frac{N}{N-1}\bar{X}^2 = 1{\cdot}076.$$

When the formula $\bar{X} \pm 1{\cdot}64\sqrt{(S^2/N)}$ is applied the true vector efficiency is found to be $0{\cdot}660 \pm 0{\cdot}234$, with a confidence of 90%. In comparing this expected vector efficiency with that of Wheeler & Douglas (1945), their data is found to be as follows: $N = 49$, $\bar{X} = 0{\cdot}43$ and $S^2 = \frac{37}{48} - (\frac{49}{48} \times 0{\cdot}43^2)$ or 0·581. Applying $\bar{X} \pm 1{\cdot}64\sqrt{(S^2/N)}$, the expected vector efficiency is $0{\cdot}430 \pm 0{\cdot}179$. Thus the intervals of vector efficiency overlap in these two experiments by different workers.

The hypothesis that the ratio of transmissions to fleas obtained for *X. cheopis* in this experiment and in that of Wheeler & Douglas differs only due to chance, is tested by using the above values and applying formula (II):

$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}\right)}} = T$$

$$\text{or} \quad \frac{0{\cdot}43 - 0{\cdot}66}{\sqrt{\left(\frac{0{\cdot}58}{49} + \frac{1{\cdot}076}{53}\right)}} = T = -\frac{0{\cdot}23}{0{\cdot}179} = -1{\cdot}28.$$

Since the computed T is within the limits of $\cdot 1{\cdot}96$ and $-1{\cdot}96$ it can then be said with a confidence of 95% that the difference in the two experiments is due only to chance.

Diamanus montanus

The experiments of Wheeler & Douglas recorded *D. montanus* to block in a minimum of 4 days, a maximum of 28 days, with a mean of 10 days. Of thirty-two fleas which blocked, ten blocked during the first week and sixteen others during the second week. The remaining six blocked between the 15th and 28th days.

Eskey & Haas recorded 53 days as the average extrinsic incubation of two blocked *D. montanus*; one blocked between the 17th and 31st days and the other between the 61st and 90th days. The fleas used in the present studies lived long enough to block according to the time given by Wheeler & Douglas, but not long enough for that recorded by Eskey & Haas. The chief difference in the techniques employed by these investigators can perhaps partially explain the difference in the extrinsic incubation period: Wheeler & Douglas fed fleas on infected mice, while Eskey & Haas used infected guinea-pigs as the host animal. Eskey & Haas obtained three transmissions with the two *D. montanus* which became infective in the total of nineteen used. The proportion of transmissions obtained to specimens used computed from their data, would be 3/19 or 0·16 for the species. The number of fleas used in these studies was not sufficient to permit strict conclusions to be drawn for the species. However, it is probably safe to assume that the strain of fleas employed by them was not an efficient transmitting strain.

The expected number of transmissions by *D. montanus* in the present studies was estimated thus:

$$N = 66, \quad \bar{X} = \tfrac{1}{66} = 0{\cdot}015,$$

$$S^2 = \tfrac{1}{65} - (\tfrac{66}{65} \times 0{\cdot}015^2) = 0{\cdot}015.$$

Applying these values in formula (I),

$$\bar{X} \pm 1{\cdot}64\sqrt{\frac{0{\cdot}01515}{66}},$$

the result is $0{\cdot}015 \pm 0{\cdot}025 = 0{\cdot}040$, or $-0{\cdot}01$, a vector efficiency of $0{\cdot}02 \pm 0{\cdot}02$, as the negative quantity cannot occur. This is the true vector efficiency with a confidence of 90%.

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The strain of *D. montanus* used by Wheeler & Douglas (1945) was originally collected from a different area than the strain used in the above experiment. Their strain came from the foothills (elevation 1000–1500 ft.) of the Sierra Nevada Mountains in Madera County, California near O'Neals. Plague has not been known to occur in this area. They obtained the following values:

$$N = 80,\ \bar{X} = \tfrac{67}{80} = 0{\cdot}84,$$

$$S^2 = \tfrac{183}{79} - (\tfrac{80}{79} \times 0{\cdot}84^2) = 2{\cdot}316 - 0{\cdot}715 = 1{\cdot}601.$$

Applying formula (I), $\bar{X} \pm 1{\cdot}64\sqrt{(S^2/N)}$ and substituting the values

$$0{\cdot}84 \pm 1{\cdot}64\sqrt{\frac{1{\cdot}601}{80}} = 0{\cdot}84 \pm 0{\cdot}232 = 0{\cdot}840 \pm 0{\cdot}232.$$

Therefore, in this experiment, the true vector efficiency for the species is $0{\cdot}840 \pm 0{\cdot}232$ with a confidence of 90%. The variation between the average number of transmissions for the species in the present studies (0·015), and the average number of transmissions per flea obtained by Wheeler & Douglas (0·84) is considerable. To test whether this difference is due only to chance, or if some factor other than chance is operating, the values obtained from the data in the two experiments are applied to formula (II):

$$\frac{0{\cdot}84 - 0{\cdot}015}{\sqrt{\left(\frac{1{\cdot}601}{80} + \frac{0{\cdot}015}{66}\right)}} = \frac{0{\cdot}825}{0{\cdot}1422} = 5{\cdot}801 = T.$$

Here T is $> 1{\cdot}96$ so the hypothesis is rejected that the two vector efficiencies differ only by chance. Some other factor or factors must therefore be responsible for this difference. The positive results of Wheeler & Douglas prove beyond question that *D. montanus* can be a good vector.

In the present studies forty-one specimens were studied in one experiment, and twenty-five in another. The first series produced one transmission, but in the second none resulted. The extrinsic incubation period, or the period before the fleas became infective, averaged 10 days in Wheeler & Douglas's experiments. Five fleas blocked in 4 days; five blocked in 5 days; three blocked in 6 days; five in 8 days; two in 9 days; and four fleas blocked in 10 days. In the present studies only two fleas blocked, though many lived long enough to block according to the extrinsic incubations obtained by Wheeler & Douglas. Since the experiments were carried out under very similar conditions it is safe to say that there was no major difference in the techniques which would be responsible for this extreme difference in vector efficiencies. It is therefore probable that the variation was due to a biological difference in the two strains of fleas studied. The fact that comparable results were obtained with *Xenopsylla cheopis* in these studies and in those of Wheeler & Douglas, both of which employed strains of fleas taken from rats in San Francisco, supports the suggestion that there may have been a strain difference in the *Diamanus montanus* collected from different localities.

Malaraeus telchinum

This species has been shown in previous experiments to be capable of acting as a vector *en masse* and would, no doubt, function efficiently in this capacity in a plague epizootic among members of the Cricetidae and Muridae, which it parasitizes. (See Burroughs, 1944.) That its ability to act as a biological vector of plague is slight is indicated both from the results of the present observations and from the studies of Eskey & Haas. The latter workers were unable to obtain a single transmission in studies employing seventy-four fleas. They demonstrate by photomicrographs a male and a female *M. telchinum* which were blocked. The prominence of the proventriculus and the oesophagus in the male, and the distended oesophagus in the female are characteristic of the phenomenon. Both of these fleas developed blocks rapidly after the infecting blood meal, the male dying within 5 days and the female within 10 days after that feeding.

In the present studies it is interesting to note that all five transmissions were obtained with fleas which were not fed for some time after the infectious meal. Three transmissions were obtained from a group of fleas which were starved 4 days after infecting them, the other two from fleas held without feeding for 5 days after infecting. The ratio of total specimens to transmissions was 5/115 or 0·043. It has been shown that infrequent blood meals aid, rather than hinder, the multiplication of bacteria in the alimentary tract. Two of the chief aids to this multiplication probably are: (1) a reduction of the flushing action which accompanies frequent feedings, and (2) a concurrent loss of phagocytic action with the appearance of fresh leucocytes in the stomach.

Orchopeas sexdentatus sexdentatus

The fifteen fleas which blocked did so in an average of 7 days after the infectious meal. The shortest time required for blocking to occur was 3 days, the longest, 16 days. The average length of life after blocking was 5 days, the minimum, 1 day, and the maximum, 18 days. The flea which survived 18 days after first having blocked on the 3rd day, broke its block at the following feeding and was never again demonstrated to be blocked. Of these fleas 70% retained their infection to death. Applying formula (I) to these data, the true vector efficiency is found to be $0{\cdot}170 \pm 0{\cdot}138$.

Eskey & Haas, studying this species, obtained only four transmissions by eighty-one specimens.

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They found that fleas which had transmitted plague to an animal survived an average of 3·7 days after transmission. The average extrinsic incubation period for three infective fleas was 38 days. The species does not approach the vector efficiency that its blocking potential would seem to indicate it has. Although the same number of fleas was studied in this experiment as in that with *Xenopsylla cheopis*, twenty-six less transmissions occurred, while only ten less blocked feedings were noted.

Nosopsyllus fasciatus

The average blocking time for this species was 16·6 days with a minimum of 5 days and a maximum of 30 days. Survival time after first blocking averaged 4 days, with a minimum of 1 day and a maximum of 16 days. Of these fleas 42% were infected when they died. Eskey & Haas observed an average extrinsic incubation period of 41 days and an average survival of 4·3 days after the first transmission. They obtained seventeen transmissions with ten infective fleas from the lot of fifty-one studied, for a proportion of 0·33. It must be remembered, however, that they occasionally fed an infective flea on several animals in a single day. Thus they found it to be almost as good a vector as *Xenopsylla cheopis*. In the present studies there were only ten transmissions by six fleas from the group of forty-eight studied, giving a ratio of 10/48 or 0·208. Applying formula I to the data obtained in these studies, the vector efficiency is seen to be $0{\cdot}213 \pm 0{\cdot}157$.

Opisodasys nesiotus

This species exhibited an unusually short extrinsic incubation period, averaging 7·7 days with a minimum of 2 days and a maximum of 24 days. The average survival time after first blocking was 4·66 days with a minimum of 1 day and a maximum of 17 days. The number of daily blocked feedings indicated a transmission potential much higher than the actual number of transmissions which occurred, as in the case of *Orchopeas sexdentatus sexdentatus*. Of these fleas 54%, over half of which had never blocked, were still infected at death.

Echidnophaga gallinacea

The reasons why the results obtained for this species are not comparable to those obtained for other species have been discussed (see pp. 379, 386). Although it was determined that approximately 80% of the fleas fed during the time of bacteraemia, the vector efficiency was calculated on the basis of all fleas used in the experiment. This constitutes still another reason for not comparing this data with that of other species, for all other fleas studied were known to have had a blood meal during the time of intense bacteraemia. Applying formula (I) to the data obtained for this species, the vector efficiency is found to be $0{\cdot}250 \pm 0{\cdot}115$ with confidence of 90%.

Oropsylla idahoensis

Eskey & Haas observed this flea to block and they show photomicrographs of two female fleas which were blocked beyond question. None of the fifteen fleas which they employed in transmission studies transmitted, however. Results of the present studies have established that *O. idahoensis* may serve as a vector mechanically, *en masse*; none of the sixty-one fleas studied individually was observed to block, however, and none transmitted. Furthermore, this flea displayed a marked ability to clear itself of an infection, only four of the sixty-one fleas, or 6·6%, being infected at death. This flea, while known to block and therefore a potential biological vector, can be assumed to be a very poor vector of plague.

Pulex irritans

Better results might have been obtained with this species had a strain adapted to rodents or some other smaller animal been used. For innumerable generations this strain has lived on deer. This flea also manifested vector capacities, as one flea blocked. Girard (1943) reports that *P. irritans* is incapable of blocking, and adds that experimentation has amply confirmed this fact.

These observations should further stress the fact that variation between strains of fleas is significant; and that only in rare instances can the vector efficiency of a species be determined with certainty from the results of an experiment with a single strain of fleas. Although only one flea was observed to block, this strain of *P. irritans* did show that it was capable of blocking.

Megabothris abantis

Mass transmissions with small numbers of fleas occurred very readily when the natural host was used. It was interesting to note that the first transmission with a group of twenty-seven infected fleas took several days longer to kill the first *Microtus* placed in the crock than the second. Some of the fleas had blocked by the time the second animal was placed in the crock and the transmission was biological rather than mechanical.

The fact that one is likely to err in claiming a species of flea incapable of serving as a biological vector upon the basis of a single experiment with approximately fifty fleas fed individually was borne out in studies with this species. Although no transmissions were obtained in studies with forty-eight fleas, and only one flea blocked, later studies with twenty-seven fleas showed eight fleas to block, and those eight were blocked on a total of twenty-one attempted daily feedings. The eight blocked fleas did not all transmit, but two of them transmitted two times each. *Megabothris abantis* is undoubtedly capable of serving as a vector of plague in nature.

CONCLUSIONS

(1) Though a satisfactory technique by which estimates can readily be made has not yet been devised, studies on the number of organisms present in the regurgitant of an infective flea indicate that it may be at times between 11,000 and 24,000 organisms.

(2) The primary stage of infection produced by an infective flea is frequently bacteraemia. This conclusion was reached when it was found that after an infective flea had fed on a mouse and the skin biopsied around the bite wound immediately afterwards, the mouse, nevertheless, contracted plague and died.

(3) It is possible to recover only a small percentage of plagué organisms when triturated with mouse skin and connective tissue, then plated on a sensitive medium.

(4) Species of fleas which are feeble biological vectors of plague, or experimentally incapable of serving as such, may, nevertheless, be efficient mechanical vectors.

(5) During an epizootic of plague in a susceptible animal population, mechanical transmission is undoubtedly of paramount importance.

(6) The vector efficiency of a species can be designated numerically within a certain range and may vary slightly within a few tenths of a unit for different experiments with the same strain of the species.

(7) Using strains of the same species collected from one limited geographical area, it is possible for different workers to obtain vector efficiencies which correspond closely, by employing the method of Wheeler & Douglas for determining vector efficiency.

(8) The vector efficiency of a species may vary considerably for different strains of the species collected from widely separated geographical areas.

(9) Due to the number of blocked fleas which failed to transmit at each attempted feeding, it is likely that the experimentally determined vector efficiencies are lower than the natural vector efficiencies, for in nature blocked fleas would have constant access to a host and would bite much more frequently, increasing the chances for transmission to occur.

(10) Of the species studied under comparable conditions, *Xenopsylla cheopis* was much the best vector. *Nosopsyllus fasciatus* was better than the others, being followed in order by *Orchopeas sexdentatus sexdentatus*, *Opisodasys nesiotus*, *Megabothris abantis*, *Malaraeus telchinum* and *Diamanus montanus*. *Pulex irritans* and *Oropsylla idahoensis* transmitted only *en masse* and not in individual transmission experiments. *Echidnophaga gallinacea* was proved to be a capable vector of plague, but as it was studied under somewhat different conditions than the other species it cannot be compared with them.

(11) The order of importance of transmission by fleas is the following:

(*a*) By the blocked flea serving as a biological vector.

(*b*) Mechanical transmission, which undoubtedly is of considerable importance during an epizootic.

(*c*) By the infected flea or its faeces being scratched into the skin.

(*d*) By the ingestion of fleas harbouring plague bacilli. Infection by the latter two methods is undoubtedly rare and of minor importance.

SUMMARY

All fleas used in this study were collected in the field and except for *Pulex irritans* were cultured in the laboratory. The ten species studied were *Xenopsylla cheopis*, *Nosopsyllus fasciatus*, *Orchopeas sexdentatus sexdentatus*, *Opisodasys nesiotus*, *Megabothris abantis*, *Malaraeus telchinum*, *Diamanus montanus*, *Echidnophaga gallinacea*, *Pulex irritans* and *Oropsylla idahoensis*.

All fleas transmitted in individual feeding studies with the exception of the latter two species, which transmitted *en masse*. *Echidnophaga gallinacea* could not be fed periodically as were the other fleas because of its tick-like feeding habits. Consequently, the vector efficiency obtained for this species is not strictly comparable to that found for the other species.

The transmission data obtained from individual flea feeding studies was analysed statistically to estimate the expected number of transmissions per flea of each species. These values are obtained as intervals which have a 90% probability of containing the true value. The true vector efficiency of *Xenopsylla cheopis* was found to be $0{\cdot}660 \pm 0{\cdot}234$ (expected transmissions per flea), that of *Nosopsyllus fasciatus* to be $0{\cdot}213 \pm 0{\cdot}157$, and that of *Orchopeas sexdentatus sexdentatus* to be $0{\cdot}170 \pm 0{\cdot}138$. *Opisodasys nesiotus*, *Megabothris abantis*, *Malaraeus telchinum* and *Diamanus montanus* transmitted very inefficiently.

Experimental evidence was obtained that different strains of a species of flea may differ markedly in their biological vector capacity. In contrast to results obtained in this study, Wheeler & Douglas found *Diamanus montanus* to be an exceptionally good vector; in their studies it proved to be an even more efficient vector than *Xenopsylla cheopis*. The strain of *Diamanus montanus* employed by them came from an area widely separated from that in which the strain used in the present studies was originally collected.

Since many blocked fleas did not transmit it is probable that the experimentally determined vector

efficiencies are lower than they would be in nature, where the blocked flea has constant access to a host and hence greater opportunity to feed.

Attempts to determine the number of organisms regurgitated by a blocked flea during its attempt to feed did not prove entirely satisfactory, but gave an indication that the number may be at times from 11,000 to 24,000 organisms. The technique consisted of feeding a blocked flea on the shaved abdomen of a mouse (later on the ear), then immediately doing a biopsy on the area around and including the bite wound. This biopsy material was then finely ground and plated on a sensitive bacteriological medium. Some mice upon which biopsies were performed nevertheless contracted plague and died. This must lead to the conclusion that an infective flea may deposit organisms directly into the capillaries and that the primary stage of infection resulting from a flea bite is frequently bacteraemia.

REFERENCES

BACOT, A. W. (1915). Further notes on the mechanism of the transmission of plague by fleas. *J. Hyg., Camb.*, Plague Supplement **4**, 774–6.

BACOT, A. W. & MARTIN, C. J. (1914). Observations on the mechanism of the transmission of plague by fleas. *J. Hyg., Camb.*, Plague Supplement **3**, 423–39.

BLANC, G. & BALTAZARD, M. (1942). On the mechanism of the transmission of plague by *Xenopsylla cheopis*. *C.R. Soc. Biol., Paris*, **136**, 646–7.

BROOKS, R. ST JOHN (1917). The influence of saturation deficiency and of temperature on the course of epidemic plague. *J. Hyg., Camb.*, Plague Supplement **5**, 881–99.

BURROUGHS, A. L. (1944). The flea *Malaraeus telchinum* a vector of *P. pestis*. *Proc. Soc. Exp. Biol., N.Y.*, **55**, 10–11.

BYTCHOV, V. A. (1935). On the role of fleas in the conservation and propagation of plague bacilli (Assistants dissertation). Parasites, transmetteurs, animaux vénimeux. *Recueil des travaux dédié aux 25me anniversaire scientifique du Professor Eugène Pavlosky, Moscou*, pp. 89–126. French summary, pp. 126–8.

CLEMOW, F. G. (1900). Remarks on plague in the lower animals. *Brit. Med. J.* **1**, 1141–6.

DE LA BARRERA, J. M. (1939). Contribucion al conocimiento de la peste selvatica en la Argentina; caracteres de brote de Mendoza en 1937. *Rev. d. Inst. bact.* **8**, 431–54.

DOUGLAS, J. R. & WHEELER, C. M. (1941). Sylvatic plague studies. I. A convenient individual mouse jar. *J. Infect. Dis.* **69**, 29–31.

DOUGLAS, J. R. & WHEELER, C. M. (1943). Sylvatic plague studies. II. The fate of *Pasteurella pestis* in the flea. *J. Infect. Dis.* **72**, 18–30.

ESKEY, C. R. (1938). Fleas as vectors of plague. *Amer. J. Publ. Hlth*, **28**, 1305–10.

ESKEY, C. R. & HAAS, V. H. (1940). Plague in the western part of the United States. *Bull. U.S. Publ. Hlth Serv.* no. 254, pp. 1–83.

EVSEEVA, V. E. & FIRSOV, I. P. (1932). The suslik fleas as reservoirs of plague bacilli during the winter. *Vestn. mikr. epidemiol. parazitol.* **11**, pp. 281–3. English summary, p. 283.

FOURIE, L. (1938). The endemic focus of plague. *S. Afr. Med. J.* **12**, 352–9. Referred to by Meyer (1942).

GAUTHIER, J. C. & RAYBAUD, A. (1902). Sur le rôle des parasites du rat dans la transmission de la peste. *C. R. Acad. Sci., Paris*, **54**, 1947.

GAUTHIER, J. C. & RAYBAUD, A. (1903). Recherches expérimentales sur le rôle des parasites du rat dans la transmission de la peste. *Rev. Hyg. Police Sanit.* **25**, 426–38.

GEORGE, P. V. & WEBSTER, W. J. (1934). Plague inquiry in the Cumbum Valley, South India. *Indian J. Med. Res.* **22**, 77–103.

GIRARD, G. (1943). Les ectoparasites de l'homme dans l'epidémiologie de la peste. *Bull. Soc. Path. exot.* **36**, nos. 1–2, pp. 4–41.

GOLOV, D. A. & IOFF, I. G. (1925). On the question of the role of the fleas of spermophiles in the epidemiology of plague. *Vestn. mikr. epidemiol. parazitol.* **4**, pp. 19–48. French summary, pp. 131–7.

GOYLE, A. N. (1927). Experiments on the transmission of plague by *Xenopsylla cheopis* and *X. astia*. *Far East. Ass. Trop. Med. Trans. Seventh Congr.* **2**, 35–9.

GOYLE, A. N. (1928). Experiments on the transmission of plague by fleas of the genus *Xenopsylla* (*cheopis* and *astia*) with a discussion on the flea species distribution in its relation to the incidence of plague. *Indian J. Med. Res.* **15**, 837–60.

HIRST, L. F. (1923). On the transmission of plague by fleas of the genus *Xenopsylla*. *Indian J. Med. Res.* **10**, 789–820.

HIRST, L. F. (1925*a*). Plague fleas with special reference to the Milroy Lectures. *J. Hyg., Camb.*, **24**, 1–16.

HIRST, L. F. (1925*b*). Researches on the parasitology of plague. Part II. The transmission of plague by the blood-sucking ectoparasites of rats with special reference to *Xenopsylla astia* and *X. cheopis*. *Ceylon J. Sci.*, Sect. D, Med. Sci., **1**, 167–99.

INDIAN PLAGUE COMMISSION (1906). Reports on plague investigations in India. I. Experiments upon the transmission of plague by fleas. *J. Hyg., Camb.*, **6**,425–82.

INDIAN PLAGUE COMMISSION (1907). Further observations on the transmission of plague by fleas, with special reference to the fate of the plague bacillus in the body of the rat flea (*P. cheopis*). *J. Hyg., Camb.*, **7**, 395–420.

INDIAN PLAGUE COMMISSION (1908). The mechanism by means of which the flea clears itself of plague bacilli. *J. Hyg., Camb.*, **8**, 260–5.

INDIAN PLAGUE COMMISSON (1908). On the seasonal prevalence of plague in India. *J. Hyg., Camb.*, **8**, Plague Number, pp. 266–301.

IOFF, I. G. (1941). *Problems in the Ecology of Fleas in Relation to their Epidemiological Importance.* Ordjhonikidze Regional Anti-Plague Experiment Station, Ordjhonikidze Regional Publishing House, Pyatigorsk. Translated by Mary H. Garlin, O.S.R.D. Liaison Office, New York. 133 double-spaced typewritten pages, and 332 references.

JELLISON, W. J. & GOOD, N. E. (1942). Index to the literature of *Siphonaptera* of North America. *Bull. Nat. Inst. Hlth*, no. 178, pp. 1–193.

JELLISON, W. J. & KOHLS, G. M. (1936). Distribution and hosts of the human flea, *Pulex irritans* Linn., in Montana and other western states. *Publ. Hlth Rep., Wash.*, **51**, 842–4.

KITASATO, S. (1894). The bacillus of bubonic plague (preliminary notice). *Lancet*, **2**, 428.

LAGRANGE, E. (1926). Concerning the discovery of the plague bacillus. *J. Trop. Med.* **29**, 299–302.

LISTON, W. GLEN (1924). The Milroy Lectures, 1924. On the plague. II. The etiology of plague. *Brit. Med. J.* **1**, 950–4.

MCCOY, G. W. & WHERRY, W. B. (1909). Subacute plague in man due to ground-squirrel infection. *J. Infect. Dis.* **6**, 670–5.

MARTINI, E. (1923). *Lehrbuch der Medizinischen Entomologie*, p. 140. Jena: Gustav Fischer.

MEYER, K. F. (1938). The role of the infected and the infective flea in the spread of sylvatic plague. *V. jschr. naturf. Ges. Zürich*, **83**, Beiblatt no. 30, pp. 160–9, Festschrift Karl Hescheler.

MEYER, K. F. (1941). The ecology of plague. *Medicine*, **21**, 143–74.

MEYER, K. F. (1942). The known and the unknown in plague. *Amer. J. Trop. Med.* **22**, 9–36.

MEYER, K. F. (1943). Plague. *Med. Clin. N. Amer.*, New York Number, pp. 745–65.

MEYER, K. F. (1946). The relation of diseases in the lower animals to human welfare, *Ann. N.Y. Acad. Sci.* **47**, 429–67. (This paper is in a symposium, pp. 351–76.)

MITCHELL, J. A. (1927). Plague in South Africa. *Publ. S. Afr. Inst. Med. Res.* **3**, 97–8.

OGATA, M. (1897). Ueber die Pestepidemie in Formosa. *Zbl. Bakt.* **21**, 769–74.

ROUX, M. E. (1897). II. Sur la peste bubonique. Essais de traitement par le sérum antipesteux, à propos d'une note du Dr Yersin, médecin de 2e classe des Colonies, directeur de l'Institut Pasteur de Nha-Trang. *Bull. Acad. méd.* **37**, 3 série, pp. 91–9.

SIKES, E. K. (1931). Notes on breeding fleas, with reference to humidity and feeding. *Parasitology*, **23**, 243–9.

SIMOND, P. L. (1898). The propagation of plague. *Ann. Inst. Pasteur*, **12**, 625–87.

STEWART, M. A. (1940). Present knowledge of the status of vectors of sylvatic plague in North America. *Proc. 6th Pac. Sci. Congr.* **4**, 433–7.

THOMPSON, A. (1903). Report on a second outbreak of plague in Sydney (1902). Notation by the Indian Plague Commission in I. Experiments upon the transmission of plague by fleas. *J. Hyg., Camb.*, **6**, 425–33 (429).

THORNTON, E. N. (1933). Positions in regard to plague in the Union of South Africa and the mandated territory of South West Africa. *Quart. Bull. Hlth Org. (L. o. N.)*, **2**, 64–90.

TICKHOMIROVA, M., SAGORSKAJA, M. & ILJIN, B. (1935). Die Nager und ihre Flöhe in Steppen-Übergangs-Landstrich der Nowo-Kasansker und Slomichensker Rayons und ihre Rolle in der Epidemiologie der Pest. *Vestn. mikr. epidemiol. parazitol.* **14**, 231–53. German summary, pp. 253–4.

TUMANSKI, V. & POLIAK, I. (1931). On the preservation of plague bacilli in fleas in Suslik nests during the inter-epizootic period. *Vestn. mikr. epidemiol. parazitol* **10**, 325–6.

VERJBITSKI, D. T. (1908). The part played by insects in the epidemiology of plague. *J. Hyg., Camb.*, **8**, 162–208.

WHEELER, C. M. & DOUGLAS, J. R. (1941). Transmission studies of sylvatic plague. *Proc. Soc. Exp. Biol., N.Y.*, **47**, 65–6.

WHEELER, C. M. & DOUGLAS, J. R. (1945). Sylvatic plague studies, V. The determination of vector efficiency. *J. infect. Dis.* **77**, 1–12.

WHEELER, C. M., DOUGLAS, J. R. & EVANS, F. C. (1941). The role of the burrowing owl and the sticktight flea in the spread of plague. *Science*, **94**, 560–1.

WHERRY, W. B. (1908). Plague among the ground squirrels of California. *J. Infect. Dis.* **5**, 485–506.

WU, LIEN-TEH (1934). The original home of plague. Manchurian plague prevention service. Memorial Volume, 1912–32. *Rep. Nat. Quarantine Serv.* pp. 143–58. 2 Peking Road, Shanghai.

WU, LIEN-TEH & POLLITZER, R. (1932). A new survey of plague in wild rodents and pneumonic plague. *Rep. Nat. Quarantine Serv.* pp. 83–201, Series III, 2 Peking Road, Shanghai.

YERSIN, A. (1894). La peste bubonique à Hong Kong. *Ann. Inst. Pasteur*, **8**, 662–7.

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