SYLVATIC PLAGUE STUDIES

IV. INAPPARENT, LATENT SYLVATIC PLAGUE IN GROUND SQUIRRELS IN CENTRAL CALIFORNIA

K. F. MEYER, R. HOLDENRIED, A. L. BURROUGHS AND E. JAWETZ

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco

The problem of sylvatic plague in its relationship to human bubonic plague is deeply rooted in the epizootics among wild rodents, ground squirrels and prairie dogs in particular. Long and Wherry\(^1\) as early as 1908, in the course of their investigations dealing with human plague in rural areas, noted that in the environs of farm houses where plague had occurred, the “digger” squirrels frequently were being decimated by sickness and death. Provided the special Plague Survey Crews of rodent hunters arrived in an area during the progress of an epizootic among the young rodents, carcasses with anatomical lesions indicative of plague as a rule were encountered without difficulty. Pasteurella pestis was isolated by cultures or animal inoculation tests. When the surveys were delayed until late summer or early fall, visibly infected squirrels were rarely detected, despite evidences of mass mortalities in the form of a markedly reduced density of population and the presence of skulls and skeletons. In an area proved to harbor plague-infected animals, it is customary to diminish the rodents by poisoning or shooting them. It is not surprising, therefore, that during the few years just following the epizootic no diseased squirrels can be found by methods depending solely on the detection of gross anatomical lesions. Based on such cursory surveys about 25 years ago, the prediction was ventured that plague had been eradicated from California.

The observations of the past decade, however, have disclosed the important fact that in many areas where infected squirrels had been discovered previously, the epizootics reappeared on the same ranch at periodic intervals (for details consult Meyer\(^2\)). It was furthermore recognized that during the interepizootic periods, the presence of plague in the region could be detected in the arthropod vectors. The sampling of fleas removed from squirrels living in plague-infected areas, and the testing of them by injecting their ground-up bodies into guinea pigs is now recognized as the method of choice for detecting the existence of sylvatic plague.

This newer methodology has been of assistance also in disclosing, with increasing frequency, plague areas of astonishingly wide geographic distribution. For example: during 1942, while ranches and sections of counties were being surveyed because of their proximity to military installations, hunting crews discovered a widespread plague epizootic among the Belding squirrels (Citellus beldingi) in the sagebrush-covered high plateaus of Lassen County. Other localized outbreaks of plague were encountered among the chipmunks (Eutamias sp.) and golden-mantled ground squirrels (Citellus lateralis) in Mono County, and among the antelope ground squirrels (Citeillus leucurus) in the desert regions near Needles in San Barnardino County.

Received for publication May 4, 1943.
Aided by grants from the Columbia Foundation.

It is well known that epizootics of sylvatic plague occur not only at shorter intervals of from 2 to 4 years but sometimes after a quiescent period of from 8 to 14 years. In support of this fact was the discovery in 1942 of an active plague epizootic, with grossly infected squirrels, on two Monterey County ranches where the rodent population was annually reduced by poisoning and where there had been neither visibly diseased rodents nor infected fleas for 14 years. The factors which determine this periodicity are not understood, but it is suspected that the epizootics are bridged by *enzootic rodent plague* which operates largely in the underground burrows and is not readily subject to investigation. Occasionally chance observations with pools of fleas, as those reported by Meyer and Eddie,4 furnish proof that plague persists indefinitely in an area once invaded, and that under ordinary conditions the gross anatomical examination of squirrels or other rodents may fail to detect its existence. Although the greatest danger to the inhabitants of sylvatic plague regions becomes manifest during the epizootics, there is little doubt that the problem of the suppression of these periodic exacerbations depends on an understanding of the endemicity during the interepizootic periods. Doubtlessly, the ultimate liquidation of the endemicity is impossible, despite the views expressed by casual observers that it can be accomplished if large sums of money are available for surveys and poison. Those familiar with the ecology of rodents and their ecto-parasites cannot share this optimism. There is need for an earnest desire to investigate and know the factors which are responsible for the preservation and the smoldering of the plague agent and how it can be held in control. The mechanism described as the persistence or perpetuation of rodent plague, or merely the "carry-over" from one season to another, has received some attention both in Russia and in South Africa. Two possibilities have been considered. The plague bacillus is preserved (a) in the body of the rodent or (b) in the infected flea. A critical analysis of the evidence available to date incriminates the flea as the all-important factor. The discovery, however, of so-called "latent" plague (a type of plague which infects squirrels without visible lesions) by Meyer in the United States, by Grikurov,5 Tumanski6 and others in Russia, and by Pirie7 in South Africa suggests the probability that these forms of infection might contribute to the persistence. The Russian investigators advanced the hypothesis that plague bacillus carriers of this kind when subjected to adverse environmental influences may develop relapses followed by a septicemia which gives the fleas an opportunity to become infected. These statements suggest a too liberal interpretation of work in which there are a great many gaps. Further observations are needed to assess the importance of the latent state in plague. Concerning the frequency or the extent of this stage of infection no accurate data are available, since it is obviously impractical to test by guinea pig inoculations the organs of every squirrel shot in the course of the surveys. It is fortunate, however, that during 1942 significant observations concerning inapparent plague were obtained in a small area in Central California.


INVISIBLE PLAGUE AT THE CALAVERAS RESERVOIR

For the past 3 years a population study of the ground squirrel (*Citellus beecheyi*) has been in progress at the Calaveras Reservoir, located on the boundary line of Alameda and Santa Clara Counties. Each year (1940, 1941 and 1942) from April to the end of November, detailed observations have been made on the density of population, movement, etc. of the rodents (for details see Evans and Holdenried, 1943).

During these periods 1,203 squirrels have been captured with live traps, 995 of them having been marked and released for continued study.

It is well known that the ground squirrel has a short, intense breeding period. At Calaveras Reservoir nearly all the young are born either the latter part of April or the early part of May. Usually by the end of May or June they leave their nests and appear above ground for the first time. This influx of young squirrels invariably increases the density of population about threefold, and changes are recognized readily even without sample trappings.

Early in June, 1942, a cursory field survey indicated that a smaller number of squirrels was present on the Dam (Area VIII on fig. 1) than during the 2 preceding years at the same time. Since rodent hunters of the California State Department of Public Health had shot a squirrel with plague lesions in the course of a routine survey of the Calaveras Reservoir Area in 1933, it was suspected that the diminution of the rodent population was in some way associated with the activities of *P. pestis*. This possibility had been anticipated in making the selection of the region for the population studies. Although in 1940 and 1941 a number of squirrels had been collected and carefully autopsied, no plague infection was discovered. Likewise, the inoculation of several small pools of fleas removed from these and other rodents caught in the population study area had yielded no positive findings. It was proved, however, that the squirrels collected from the Calaveras Dam area possess a greater resistance to an artificial intracutaneous injection of a California strain of *P. pestis* than those secured from a supposedly non-infected area. In a group of 104 squirrels from the endemic area, which had been injected with from 1,100 to 30,000 plague bacilli, 39, or 38% developed fatal bubonic plague. Of 268 rodents from the non-endemic area injected with the same dosage of plague bacilli, 153, or 57%, succumbed to plague. Identical experiments conducted by McCoy (data presented herein) indicate that the percentage of squirrels resistant to artificial infections with *P. pestis* is higher among those taken from localities where epizootics have prevailed than among those derived from regions in which the disease has not been demonstrated. Similar observations concerning the resistance of rats in endemic and non-endemic areas have been placed on record by McCoy and Sokhey and Chitre. It is important nevertheless to recall that resistant rodents may be encountered in regions proved free from plague, and it is therefore inadvisable to judge or to conclude a priori from the mortality rates of a limited number of experimentally infected rats or squirrels as to whether plague had or had not existed there in the past. How this resistance may be acquired is not known; it may be that it is acquired through a clinical or a subclinical infection. On the other hand, it may depend on a process of...

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natural selection, the susceptible rodents succumbing while the resistant ones survive. The offspring of the latter may possess an innate genetic resistance.

In order to secure definite information concerning the cause or causes of the reduction in the rodent population at Calaveras Dam, collections of fleas were instituted. Between June 17 and 26, 1942, 99 squirrels were trapped, and approximately 2,400 fleas, nearly one-half of which were *Diamanus montanus*, were collected. Since the collection was made in one of the trapping areas reserved for the population study, none of the squirrels were killed. In fact, an effort was made to disturb them as little as possible. The rodents were forced from the traps into mason jars, and lightly anesthetized with chloroform in order to kill the fleas. They were then placed on aluminum pans carefully combed and thus defaunated. The arthropods (fleas and ticks) were placed in normal sterile salt solution with gentian violet in a dilution of 1:70,000, and the vials were held in the refrigerator until the fleas were tested. The squirrels were released after their identity had been recorded. These procedures allowed some of the squirrels to be captured and their fleas removed several times during the period in which samples were taken. During the month of June the flea collections were taken from 80 different individual squirrels in the course of 99 captures.

![Image](https://academic.oup.com/jid/article-abstract/73/2/144/823051)

**Table 1.**—Pools of Fleas Collected in Area VIII at Calaveras Reservoir Producing Plague in Guinea Pigs

<table>
<thead>
<tr>
<th>Pool Numbers</th>
<th>Date (1942)</th>
<th>Number of Fleas</th>
<th>Number of Squirrels</th>
<th>Guinea Pig Test Inoculation Autopsy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>June 17</td>
<td>?</td>
<td>8</td>
<td>June 25/July 4</td>
</tr>
<tr>
<td>(2)</td>
<td>* 18</td>
<td>?</td>
<td>10</td>
<td>* 25/ 6</td>
</tr>
<tr>
<td>(3)</td>
<td>* 18</td>
<td>210</td>
<td></td>
<td>* 25/ 3</td>
</tr>
<tr>
<td>(4)</td>
<td>* 19</td>
<td>206</td>
<td>7</td>
<td>* 25/ 8</td>
</tr>
<tr>
<td>(5)</td>
<td>* 19</td>
<td>110</td>
<td>3</td>
<td>* 25/ 6</td>
</tr>
<tr>
<td>(6)</td>
<td>* 20</td>
<td>228</td>
<td>9</td>
<td>* 25/ 2</td>
</tr>
<tr>
<td>(10)</td>
<td>* 25</td>
<td>236</td>
<td>11</td>
<td>July 9/ 13</td>
</tr>
<tr>
<td>(11)</td>
<td>* 26</td>
<td>187</td>
<td>9</td>
<td>* 9/ 14</td>
</tr>
</tbody>
</table>

*All guinea pigs showed typical lesions of plague at autopsy.*

The 2,400 fleas were divided into 11 pools, each consisting of approximately from 110 to 280 fleas. Each pool of insects was carefully rinsed with salt solution, and the fleas were carefully triturated in a mortar with a few cc of salt solution. The suspensions were inoculated subcutaneously into guinea pigs. The pertinent data are summarized in table 1. *Eight of the 11 pools produced typical fatal plague infections.* The inoculated animals died from plague within 7 to 13 days after the injection. Since com-
The Calaveras Dam region was scrutinized for evidences of dead squirrels. Openings of burrows were inspected for skulls and skeletons, or for the presence of flies usually indicative of decomposing cadavers. In Area VI a small number of burrows, which appeared to be unoccupied, were opened up and the nesting places explored. No carcasses or carrion were found.

Guinea pigs held in suitable cages were placed within the openings of a few burrows which appeared to have been recently abandoned but which still showed numerous fleas when cotton batting was exposed at the rim. It had been proved previously that these sentinels would serve as hosts for the squirrel fleas. If a guinea pig thus exposed succumbed to a plague infection, it would furnish indirect evidence of the presence of infective fleas. None of the guinea pigs exposed in Area VIII became infected with plague.

The field surveys and inspections of the burrows yielded no evidence of an active epizootic. It was imperative to determine the extent of the infection which had given the fleas an opportunity to ingest *P. pestis*. With the kind assistance of Mr. E. T. Ross, Chief of the Bureau of Sanitation, State Department of Public Health, a Survey Crew was delegated to assist in the investigations. At regular intervals for a period of 4 months, the crews shot squirrels in the area surrounding the Reservoir, killing 259 in July and 117 in August, most of them young but some old. The locations are indicated as Area I to Area VII on map 1. The uneven density of the rodent population prevented the collection of identical samples without causing profound disturbances in the squirrel populations, which might seriously have interfered with the surveys in subsequent months. In fact, the presence of relatively few squirrels in Areas III, IV, V and VI prevented collections during September.

The cadavers of the squirrels were dissected carefully and examined for plague lesions. No gross anatomical lesions generally considered indicative of plague were observed. Although the lymphatic nodes frequently were enlarged, inflammatory reactions or suppurative lesions were not discernible on either gross or microscopic examination. Occasionally the spleens of some of the squirrels were enlarged, but little significance could be attached to these findings because in previous years the hypertrophy had been found to be associated with the presence of a trypanosome, *Trypanosoma cruzi* variety, probably transmitted by the triatoma (*Triatoma protracta*), not infrequently present in the rodent burrows.* In view of the negative gross findings and guided by previous observations made in other sections of the State in connection with "latent" plague, it was decided to collect routinely the organs of the squirrels for guinea pig inoculation tests. The spleen, liver, and the iliac, axillary and inguinal lymphatic nodes from each squirrel were placed in sterile containers and promptly iced. As a rule, the tissues from 4 to 5 squirrels were pooled, carefully pulped in a mortar and suspended in salt solution to a concentration of from 10 to 20%. Cultures prepared from the suspensions yielded *E. coli* in varying concentrations. Previ-

* In July and August, 1940, several young squirrels brought to the laboratory and held under observation lost weight. When sacrificed, they showed marked anemia as well as the splenic enlargement. Blood films revealed the trypanosomes, about 1 in every 20 fields, containing a large oval kinetoplast and morphologically resembling the trypanosomes discovered in California by Kofoid and Whitaker* in wood rats (*Neotoma fuscipes*), and supposedly transmitted by *Triatoma*.

ous experiences, extending over many years, have shown such contaminations to be well tolerated by guinea pigs injected subcutaneously with 2 cc amounts of the suspensions. The inoculated Fifteen, or 16% of the pools induced fatal plague-infections in guinea pigs. Survey Area VI, located about three-quarters of a mile from Dam Area VIII, where infected fleas had been found, and

guinea pigs have been observed until death or the survivors for 30 days.

The important results which accrued from these inoculation tests are summarized in table 2.

A total of 94 pools, consisting of pulped organs from 440 squirrels, were tested during a period of 4 months, the adjacent Study Area IX, in which no shooting was done in order that the rodent population would not be disturbed, yielded the largest number of positive pools. Twelve, or 35% of 34 organ pools infected the inoculated guinea pigs. Plague was likewise present on the opposite side of the Reservoir
in Area III, and at the lower end of the Reservoir in Area I which is located approximately 3 miles from the Dam (Area VIII). With the exception of one pool, encountered in August in Area VI, latent plague was demonstrable only in the organs of squirrels shot during the month of July. For ecological reasons, and with the intention of preserving the rodent population for further studies.

_P. pestis_ differed in no way from those encountered in the fleas, or in many field specimens collected in California and proved infected in 1942. On the other hand, the last pool of the series, prepared on August 26, produced typical lesions in the guinea pig, which died on the 21st day after inoculation.

Fleas present on the squirrels shot by the crews were placed in salt solution.

Collections of arthropods were also made at the opening of the burrows and from squirrels which were trapped in Areas VIII and IX. The insects collected during July were identified, and separate pools consisting of from 10 to 80 individuals of one species of squirrel fleas, either _D. montanus_ or _Hoplopsyllus anomalus_, were tested on guinea pigs. Later in the season, composite samples of from 150 to 300 fleas were used for the inoculation tests. Through the laboratory studies of Wheeler and Douglas, it was known that _Hoplopsyllus anomalus_ is probably a poor transmitter. By testing pools of separate species, an attempt was made to find this species in an infected state in nature. The examinations dealing with the inoculation of flea pools are summarized in tables 2 and 3.

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The fleas collected from squirrels that had been shot and dissected by the Survey Crews were tested in 86 pools, which failed to infect guinea pigs. Equally disappointing were tests made with fleas assembled from various sources during the months of July to November in Area VIII, where infected fleas had been encountered during the latter part of June. The 130 pools, representing a collection of 17,045 fleas over a period of 6 months in an area of less than 20 acres, apparently contained few if any plague bacilli capable of infecting guinea pigs.

For the present, no satisfactory explanation can be offered for the failure to detect plague-infected fleas in the 68 pools, which were assembled from fleas collected in July in the area where only a few weeks before 8 of 11 pools were found to cause plague in guinea pigs. It is only proper to consider the various possibilities which might have contributed to the sudden disappearance of infected fleas.

One thought to be seriously considered is that the exposure of the fleas to room temperature, while they were being examined and identified under the microscope, might have damaged the few plague bacilli present in the fleas permeated by salt solution. Vials with fleas were frequently kept on the working benches for many hours at high temperatures.

Although the flea index on the individual squirrel, as shown in table 4, remained of the same magnitude during the test period and consisted of from 24 to 55 fleas per rodent late in June and early in July, a shift in the species occurred. A glance at fig. 2 shows that in July each squirrel carried from 9 to 14

**Table 4.—Summary of Flea Population Density on Squirrels of Area VIII**

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of Flea Collections</th>
<th>Number of Fleas per Squirrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>99</td>
<td>2,400</td>
</tr>
<tr>
<td>July</td>
<td>264</td>
<td>8,624</td>
</tr>
<tr>
<td>August</td>
<td>105</td>
<td>5,106</td>
</tr>
<tr>
<td>September</td>
<td>71</td>
<td>1,864</td>
</tr>
<tr>
<td>October</td>
<td>102</td>
<td>3,091</td>
</tr>
<tr>
<td>November</td>
<td>63</td>
<td>3,462</td>
</tr>
</tbody>
</table>

**D. montanus**, and from 22 to 25 *H. anomalus*. In fact, this shift in the species distribution already observed by Stewart and Evans 14 in 1940 was even more marked in August. The heat-tolerant, but inefficient plague-vector, *H. anomalus*, was just as common as the *D. montanus* which, however, remained fairly constant in number—about 10 to 14 individuals on each squirrel. Since each species of fleas removed from a number of squirrels was pooled separately into lots of from 50 to 200 insects, an adequate sample of *Diamanus* was tested for plague bacilli during the month of July. In the future another hypothesis must be tested. It is not unlikely that during the hot weather infected fleas may remain in the depths of the cool burrows; they are not brought to the surface in the hair of the squirrels and thus are excluded from the customary test pools.

Another possibility which must not be ignored is that the study merely caught the end of an epizootic which operated among the young squirrels shortly after

they were born and arrived on the surface. The infected fleas found late in June may have received their infective meal from these rodents.

Since only squirrels with "closed" inapparent plague were encountered in the course of the shooting surveys, the fleas had no opportunity to become infected. Other field surveys, and in particular special studies at higher altitudes by Evans, Wheeler and Douglas in Kern County (1941), have shown that no infected fleas were found after July 18 in an area where a visibly destructive plague epizootic had operated from April to June. In this connection, it must be recalled that squirrels with latent plague were found in this particular region as late as October, at least 3 months after the last infected fleas had been demonstrated. The observations made at the Calaveras Dam resemble those recorded for 1941 in Kern County. They strongly indicate that any sylvatic plague epizootic among squirrels, whether apparent or inapparent, coincides fairly well with the period of dispersal of the young squirrels. Studies concerning the flea-squirrel infection chain must be concentrated during the months of May and June at the lower elevations.

It must be recognized that the foregoing explanations and interpretations are incomplete, and probably applicable only to conditions as they prevailed at the Dam in 1942. The ecological factors observed in one area may not have been

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in operation in another region. The infection may become occult in one aggregate of colonies, while apparently continuing to operate in another. For example, on September 12, 1942 the State Survey Crew, hunting 2 miles south of Pleasanton (13 miles from the Calaveras Reservoir), shot 9 squirrels and collected 70 fleas from them. When inoculated as a pool, these fleas produced plague in guinea pigs. While frank infections are found in the squirrels, the fleas have an opportunity to become infected and allow plague to persist for some time after the epizootic has been in operation. Although it is merely a surmise, this was probably the case in the Pleasanton area. The gross examination of the cadavers of the rodents did not suggest the existence of plague, and no tissues were sent to the laboratory. In order to fill out the various gaps which have been brought to light in the course of these studies, it will be worth while to test the fleas or organ pools of other rodents (Microtus, Peromyscus, Neotoma, etc.) while the surveys are being made. In fact, recent observations leave no doubt that the examination of fleas and organ pools of Microtus is very important.

For the sake of economy in time, effort and experimental test animals, little attention has been paid to the various species of Cricetidae and Muridae which inhabit the epizootic or enzootic plague areas. Sporadic sampling has disclosed intriguing findings. In 1937 and 1938, pools of organs prepared from apparently normal mice (Peromyscus sp.), collected early in October, produced plague in guinea pigs; in 1942, late in October, the gross anatomically normal organs of Microtus yielded P. pestis on inoculation of guinea pigs. Infected fleas were removed from wood rats in Nevada and Utah in 1938 (see Eskey and Haas16) and from mice (Microtus) in September, 1942. An epizootic sylvatic plague area in Monterey County had produced many pools of infected fleas and diseased squirrels. In 1942 the burrows in this area were intensively treated with carbon bisulphide and thoroughly covered with poison. In addition to this, the area was freed of squirrels by shooting during a period of six months. During March, 1943, the organs of 10 Microtus californicus, and 37 fleas from 16 M. californicus infected guinea pigs with plague.

The remarkable similarity of the ecology of sylvatic plague in southeast Russia and South Africa, in which the rôle of house mice (Mus musculus) and multimammate mice (Mastomys coucha) in the African Kraal is fully established, forcibly indicates that the position of the Muridae and Cricetidae in the rise and fall of plague epizootics among squirrels must receive serious consideration.

It may appear exceedingly speculative to consider the possibility of the plague bacillus undergoing deterioration in virulence while resident in the organs of squirrels. Until a few years ago, it was generally believed that strains of plague bacilli recently isolated from infected human beings and animals invariably were capable of causing fatal infections in guinea pigs. From their experience in New Orleans in 1922, Williams and Kemmerer17 concluded that towards the end of an epizootic there is an increase in the number of rats without lesions that yield strains of apparently low virulence. More recently, Pirie18 reported on a noteworthy diminution in virulence of P. pestis ob-

tained in South Africa from human and animal sources. The lack of virulence has not as yet been explained, but it has greatly hampered his experimental work. Since a strain of plague bacilli is a composite mixture of virulent and avirulent organisms, environmental conditions might develop which could overbalance the growth in favor of the avirulent component. How this could have taken place within a few weeks must remain unexplained.

In the Observation Area (IX), the trapping operations furnished suggestive evidence concerning the prevalence of inapparent plague. For two years, mammalogists engaged in the population study had handled over a thousand squirrels without encountering any noteworthy abnormalities. Early in June, 1942, they noticed some of the immature squirrels had enlarged inguinal lymphatic nodes. Some of the nodes were three-quarters of an inch long and raised the skin an eighth of an inch. By the end of September, 36 of 81 immature squirrels examined presented a definite lymphadenopathy. Late in August, the lymphatic nodes of 2 squirrels were tested by guinea pig inoculation, with negative results. Microscopic sections of portions of the same nodes showed hyperplastic proliferations of the reticulum, but no evidences of focal inflammation or productive processes in the perivascular tissue. Cultures yielded no microorganism.

A comparison of the weight curves obtained for the immature squirrels during 1940, 1941 and 1942 disclosed noteworthy differences. The essential data are summarized in fig. 3.

During 1940 and 1941, the weight of the rodents increased at approximately
the same rate; in 1942, however, the gain was decidedly less rapid. Starting with the average weight in June, which was about the same for the immature squirrels during the three seasons, the males, ordinarily the more rapidly growing sex, showed a smaller increase between July and August than that recorded for either 1940 or 1941. By November they averaged 500 gm, 75 gm less than the immature males of 1941, and 125 gm less than those of 1940. Until August, the average weight of the immature females—from 350 to 375 gm—was the same during the 3 years. Between August and November of 1942, however, the net weight gain was only 50 instead of 125 gm. These differences in the rate of growth established for 1942 cannot be attributed to insufficient food supply. The region has an abundant vegetation, and there has been more food available to the squirrels than they were able to eat. Thus, it is reasonable to suspect that the retarded growth is attributable to the effects of an infection. In all probability, the inapparent plague epizootic permeated the squirrel colonies on a scale similar in extent to a visible epizootic, with the exception that the infections were rarely fatal. Accurate data concerning the mortality are not as yet available, although the 1942 census figures indicate a loss in the immature squirrels. The isolated Observation Area (Area IX on map) of about 8.2 acres has been thoroughly and frequently trapped to furnish a rough census of the number of squirrels, and to indicate the main trends in the population. These fluctuations in the density of population for the years 1940 and 1941 have been graphically shown by Evans and Holdenried in a recent report. At the Calaveras Dam, at least some of the squirrels estivate. While they are inactive they cannot be trapped, and thus their failure to appear in the late summer and fall trapping records of 1942 does not necessarily prove that they are no longer part of the population. Continued trapping in 1943, after the estivating squirrels have become active, should reveal which of the squirrels have estivated and which have disappeared. The additional data will allow the construction of a population curve that can be compared with those of 1940 and 1941. Only when these data are available will it be proper to estimate the effect of the "invisible" epizootic on the squirrel population during 1942.

DISCUSSION

The observations presented in this paper add further evidence that squirrels, with no anatomical lesions suggestive of plague, may harbor \textit{P. pestis} highly infectious for guinea pigs. In 1931, Popov,\textsuperscript{19} dealing with the diagnosis of suslik (\textit{Citellus pygmaeus}) plague, pointed out the great difficulties which beset this work. The usual practice, in the absence of macroscopic changes, of relying upon the microscopic examination of organ smears was faulty, because the absence of gross signs and negative smears did not exclude plague. On the other hand, some blood parasites of squirrels cause alterations similar to those of plague. To obtain further insight into this problem, Popov dissected 608 \textit{pygmaeus} in an enzootic region. In each case agar cultures were made from the spleen, liver, lungs, heart blood and lymph-nodes, if sufficiently enlarged. Of 13 susliks, appearing macroscopically normal, 7 yielded \textit{P. pestis} in several organs, while 2 had organisms in one organ, 2 in two, and 2 in three organs. It is likely that animal experiments with the pooled organs from the apparently normal corpses would have further improved the results. Latent

\textsuperscript{19} Rev. Microbiolog. Saratov 10: 63, 1931.
plague infection involving not less than 20% of the susliks has been seen by Konstansoff and by Syssin in tarabagans (Arctomys bobac).

There is general agreement among observers that this form of plague is in some way connected with the "carry-over" of the infection from season to season and its persistence in the enzootic areas. A definite distinction is made between latent and chronic plague. The Indian Plague Commission presented ample evidence to refute the theory that cases of chronic or "resolving" rat plague are of importance in spreading the infection. These conclusions were reached in 1905, and no satisfactory evidence has been brought to light which would justify a change of opinion. The investigators in the south Russian and Transbaikalian sylvatic plague areas were therefore forced to give their findings a different interpretation and significance. Latent or "carrier" plague in wild rodents represents a stage of survival of plague bacilli in the tissues. The absence of inflammatory or encapsulating lesions favors the free distribution of the bacilli, and the occasional bacteremias may give the fleas an opportunity to become infected. How the bacteremia, the conditio sine qua non, for the propagation of the disease among the rodents is fulfilled remains unexplained. Although experiments by Wu Lien-Teh to reproduce latent plague were in part successful, they have furnished no evidence of a bacteremia. Until more elaborate experiments have been devised to reproduce the type of inapparent plague described in this paper, it is advisable to suspend further speculations relative to its significance in the ecology of sylvatic plague.

There are, however, a number of practical implications which deserve discussion. The Calaveras records offer an explanation for the failure in 1933 to find plague bacilli by testing a total of 233 pools prepared from the organs of 1,380 rodents. These rodents had been shot or trapped in 15 counties of California where sylvatic plague had been demonstrated in previous years. The specimens were all collected late in October and November at low altitude levels. These examinations were instituted to explain the many positive pools which had been encountered in October in an area with an active epizootic in chipmunks and golden mantled squirrels. From the Calaveras data it appears not unlikely that the persistence of a latent infection in the Beechey ground squirrels is probably confined to a short period of 1 or 2 months. Random sampling on ranches where plague has existed in the past should be limited to the months of May, June and July. These months provide better chances of discovering invisible plague activity than does the late fall. When the wild rodents in a high altitude region have passed through a plague epizootic as late as August, latent infection may occasionally be discovered shortly before hibernation. In the light of the observations here reported, rodent surveys depending entirely on macroscopic examinations of cadavers, are not only incomplete, but in fact, futile. Although many examples could be cited, one is particularly significant. In 1933, while pursuing a report of a squirrel mortality in Kern County, the Survey Crews examined 430 squirrels in June and 476 in September, with entirely negative results. In March and April of the next year, however, the mortality was proved to be caused by plague (for details, see...
Evans, Wheeler and Douglas[15]. The experiments made in Kern County and in other regions suggest that inapparent infections of squirrels and likewise of mice may be the precursors to frank epizootics. This possibility has received attention, and it will be of interest to follow the behavior of sylvatic plague in the Calaveras Dam region. In fact, it is anticipated that the close study of the ecology of the rodents and their fleas in a small area will ultimately furnish a better understanding of the problem than the cursory surveys and random sampling of many regions. Such a study area should be judiciously sampled in order that the density of the rodent population is not profoundly disturbed. Many a good opportunity to collect comparative data has been ruined by the Rodent Poison Crews, who promptly invaded the territory after plague had been demonstrated. There is no need to emphasize that the crude routine of examining squirrels or other rodents for gross plague lesions, even by expert pathologists under ideal laboratory conditions instead of in the field, will not improve the results until pool testing of the organs and the systematic sampling of the fleas become obligatory. The burden which such a policy will place on the laboratory is obvious. In peaceful times, an organization could be developed consisting of well-trained mammalogists and entomologists with an outlook for research and adequate facilities, provided that the continuous support of a plague control program is recognized to be just as important as the maintenance of a fire or police department. Finally, it is anticipated that the newer testing procedures will in time supply a definite answer to the pertinent question: "How efficacious are the rodent control measures in suppressing sylvatic plague?" The evidence already at hand casts a deep shadow of doubt on the present day methods.

**SUMMARY**

Plague was discovered in an area at the Calaveras Reservoir, Alameda and Santa Clara Counties, California, where for the past 3 years a population study of the ground squirrel (*Citellus beecheyi*) has been in progress. In June, a cursory field survey indicated that a smaller number of squirrels were present than during the two preceding years at the same time. From 99 squirrels, approximately 2,400 fleas were collected and divided into 11 pools. The triturated fleas were tested on guinea pigs. Eight of the pools produced typical fatal plague infections. Field surveys and inspections of burrows yielded no evidence of an active epizootic. In fact, a very careful anatomical examination of 259 squirrels shot during July and 117 killed in August revealed no lesions generally considered indicative of plague. The spleen, liver and lymphatic nodes from 4 or 5 squirrels were pooled, pulped, suspended in salt solution and injected subcutaneously into guinea pigs. During a period of 4 months a total of 94 pools consisting of pulped organs from 440 squirrels were tested. Fifteen, or 16%, of the pools induced fatal plague infection in guinea pigs. The rodent population area in which infected fleas had been found yielded 11 positive pools in July and 1 positive pool in August. Additional organ pools were tested during September and October with negative results. Simultaneously 130 pools, representing a collection of 17,045 fleas over a period of 6 months (July to December), contained few, if any, plague bacilli capable of infecting guinea pigs. The significance and practical importance of this form of latent plague infection in its relation to inapparent or occult plague epizootics in squirrels and field mice and to future survey studies of sylvatic plague areas are discussed.