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Ecology of Plague in Vietnam I. Role of *Suncus murinus*. (31930)

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The importance of the insectivore *Suncus murinus* as a reservoir for plague has been a matter of concern for a number of years. In 1914, Kerandel(1) isolated *Pasteurella pestis* from *S. murinus* in Phnom-Penh, Cambodia. He noted that specimens trapped in this area harbored numerous rat fleas, *Xenopsylla cheopis,* and concluded that since the shrew shared fleas with the more common plague carriers that it might serve as an unrecognized reservoir.

In 1932, Kopstein(2-5) reported the results of a survey of 10,000 houses in Java. He found *S. murinus* to be a village dweller living in close association with *Rattus rattus,* both within and outside the dwellings. Approximately half of the *S. murinus* were found to be carriers of *X. cheopis*. He believed that while *S. murinus* may play a role in transmission of plague among rodents, its lack of contact with man excluded it as an important factor in human plague. Since it was found exclusively in the villages, he doubted the shrew played any part in the transmission of plague from village to village. Schuurman (6,7) disagreed with Kopstein concerning the role of *S. murinus* in the epidemiology of plague. He believed that the shrews were active migrators and as such could play a role in intervillage transmission.

Suncus murinus trapped during the 1922-1930 plague outbreak in Macassar were found infested with both *Xenopsylla astia* and *S. cheopis,* the first species predominating (Van Der Walle(8)).

Sharif and Narasimhan(9), as quoted by Pollitzer(10), stressed the importance of *S. murinus* wandering from house to house as well as undertaking excursions from village to village in India. Pollitzer further stressed their role in conveying the rat ectoparasite into human habitations.

Suncus murinus has been recognized as a potential reservoir in China by Pollitzer(11) in 1948 and by Yang *et al*(12) in 1939. In a review of plague in China by Kraminskiy(13), he reported that the shrew was often found in the plague infected villages of Fukien. *Pasteurella pestis* was isolated from 1 of 262 *S. murinus* trapped in the Yunnan foci.

Herivaux and Toumanoff(14) noted an increase of *S. murinus* in the Saigon outbreak of 1941-43. However, records of the Institute Pasteur, Saigon reveal no isolations of *P. pestis* from this species in the various surveys conducted in Vietnam.

Materials and methods. As part of the plague investigation in Vietnam, a program of animal trapping was carried out. Live box traps were set out each night in the Saigon-Cholon area and at varying intervals in other geographic areas of Vietnam. The traps were

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TABLE I. Results of Trapping in Various Areas of Vietnam.

Area and trapping period	No. of *S. murinus* trapped	% of total animals of all species trapped	No. of fleas found on *S. murinus*	Flea index	% of total fleas collected	*P. pestis* isolated from
Saigon-Cholon (11 mo)	1243	24	926	0.74	16	3 spleen pools
Nha Trang (3 wk)	210	37	1545	7.4	44	3 " " 3 flea pools
Remainder of Vietnam (11 mo)	304	9	116	0.38	5	1 spleen pool

collected each morning. In the Saigon-Cholon area and in special study areas such as Nha Trang, the captured animals were transported live to the laboratory. Upon arrival at the laboratory, the animals were bled and the blood from *S. murinus* pooled. The carcasses were combed to remove the ectoparasites which were pooled by trapping area. At autopsy, each specimen was examined for gross lesions, and the spleen removed and pooled for bacteriological examination. In the more remote areas, trapped animals were killed, placed in plastic bags, frozen, and shipped to Saigon for examination. With the exception of bleeding, all specimens were treated in the same manner as locally trapped animals.

Flea and spleen pools were separately ground with sterile sea sand in a mortar. Sterile saline was added and the tissue suspension was injected into 2 susceptible white mice. The mice were observed daily for a period of 14 days and sacrificed. All mice, those dying during the observation period and those sacrificed at the end, were autopsied. Smears were prepared from heart blood and the cut surfaces of the spleen and liver. All smears were air dried, fixed in methanol for 15 minutes, stained with Wayson's stain, and examined for bacteria.

The spleen and heart blood of all animals were cultured on desoxycholate agar and peptone agar. Cultures were incubated at 28°C and examined daily for 3 days. All suspicious colonies were picked and identified. Identification of *P. pestis* was based on the criteria recommended by Baltazard *et al*(15).

Pooled sera were tested for *P. pestis* fraction I antibody by the microhemagglutination test recommended by Cavanaugh *et al*(16).

Results. During the period from 1 July 1965 through 30 June 1966, a total of 1,757 *S. murinus* were examined for plague. None of the animals had gross lesions indicating an acute infection. These animals harbored 2,587 fleas of the genus *Xenopsylla*. Speciation was not undertaken. *Pasteurella pestis* was isolated from 7 spleen pools and 3 flea pools. Table I lists the numbers of animals trapped, the ectoparasites recovered and the number of strains isolated from different areas. The overall rate of isolation of *P. pestis* from *S. murinus* was 4 per 1,000 animals tested, which was nearly identical to the 4.4 per thousand isolation rates for *Rattus norvegicus* and *Rattus rattus* during this same time period. The isolation rate from the ectoparasites varied among the 3 species. Rates were: *R. rattus* 2.1, *S. murinus* 1.2, and *R. norvegicus* 0.9.

Comparison of data between areas in Table I is not justified as the time periods covered are not similar and the intensity of trapping was not comparable. The Saigon-Cholon area represents a continuous year-round trapping program where 25-40 traps were set 5 nights a week in neighborhoods where human plague cases had been reported.

The Nha Trang data was obtained from an intensive 3-week survey where 100 traps were set nightly in 5 different socioeconomic areas of the city during the peak of the local plague season.

The data for the remainder of Vietnam consist of a collection of results obtained during an 11-month period from August 1965 to June 1966 from diversified areas, including military camps, villages, and areas of operations. Usually no more than 10 traps were set in any location and any given area was not trapped for more than 3 consecutive nights. Human plague cases may or may not

have occurred in or near the trapped areas during the preceding weeks. The number of fleas and the corresponding flea index is low as many of the animals were caught in snap traps and the fleas had departed the dead animals prior to being placed in the plastic bags for shipment.

The results of serological examination indicate that several *S. murinus* had prior experience with *P. pestis*. Eight of 42 serum pools from the Saigon-Cholon area had antibody titers of 1:16-1:256 against fraction I of *P. pestis*. In the Nha Trang area, 7 of 13 pools reacted with titers of 1:16-1:256. The Nha Trang area will be trapped 3 additional times during the coming year in order to obtain seasonal variation. When these data are available, a comparison between the sea coast city of Nha Trang and the inland city of Saigon may be made.

Discussion. The isolation of *Pasteurella pestis* from 7 *Suncus murinus* spleen pools and 3 flea pools demonstrates the involvement of this species in commensal plague in Vietnam. All individuals represented in the positive pools were trapped in and around inhabited dwellings. Assuming that each positive pool contained one infected animal, the rate of isolation, 4 per 1,000, while low, was essentially the same as that for the recognized plague carrier species *R. norvegicus* and *R. rattus*. Less than 50 specimens were taken more than 1 kilometer from a village and all were negative for *P. pestis*. Therefore, due to this limited trapping experience in noninhabited areas, no data are available to clarify the role *S. murinus* may play in the transmission of plague from village to village.

Zeville(17) reviewed the records of the Institute Pasteur, Vietnam, and reports that in the period between 1928 and 1942, 25,000 rats were examined and 16 were found positive or a rate of 0.6 per thousand, while in the Saigon epidemic of 1943, only 3 of 7,853 rats examined were positive. He concluded the data confirmed Baltazard's(18) hypothesis that the true reservoir of infection was not the more sensitive species such as *R. norvegicus* and *R. rattus* but rather those species more adapted to the disease, that is, among the wild rodents.

While *S. murinus* is neither a rodent nor a forest dweller, it may have become sufficiently adapted to infection with *P. pestis* to serve as a reservoir for the organism. None of the individuals examined showed any signs of active infection even though *P. pestis* was repeatedly isolated from them. The degree of resistance was not determined by challenge experiments due to our inability to maintain large numbers of *S. murinus* in captivity. However, indirect serological evidence indicates a sizeable percentage of the groups tested had experience with *P. pestis*. Fifteen of 55 serum pools tested by the microhemagglutination test had titers of 1:16-1:256 against *P. pestis* fraction I. According to Cavanaugh *et al*(16), a positive titer of 1:16 or greater is evidence of past infection.

Summary. *Pasteurella pestis* was isolated from 7 *Suncus murinus* spleen pools and 3 flea pools obtained from it. Lesions indicating an acute infection were not observed in any of the 1,757 animals examined. When pooled sera were examined, 15 of 55 pools had titers of 1:16-1:256 against fraction I of *P. pestis*.

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Erythrocytic Abnormalities in Experimental Malaria.* (31931)

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In malarial infections, the osmotic fragility of both parasitized and nonparasitized red blood cells is significantly increased(1,2). We believed that measurements of the erythrocytic size, surface area, hemoglobin concentration and volume changes in hypotonic saline solutions would provide information about the effect of malaria upon red blood cells. This study reports the erythrocytic changes observed in hamsters infected with *Plasmodium berghei.*

Methods. Adult Walter Reed strain hamsters were used in these studies. For malarial experiments, hamsters were infected by intraperitoneal inoculation with 2×10^7 *P. berghei*-infected mouse erythrocytes (New York University gametocyte strain). In experiments in which acetylphenylhydrazine (APH) was used, 20 mg/kg of a saline solution of the drug was injected intraperitoneally each day for 13 days. Blood was collected from the axillary vein. Leishman-Giemsa stained blood films were prepared from fresh blood to determine the percentage of parasitized and polychromatophilic cells. Erythrocytic diameters were measured on these films with a calibrated micrometer eye piece using a simplified Price-Jones technique(3). For other studies the blood was anticoagulated with heparin and washed 3 times with normal saline. After the initial centrifugation heavily infected blood formed 2 distinct layers. In certain experiments these fractions were separated and tested individually. Mean corpuscular hemoglobin concentrations (MCHC) were calculated from microhematocrit and hemoglobin determinations.

Determinations of erythrocytic volumes were performed with the Coulter counter with a 50 μ siliconized aperture and a 25 window plotter (Coulter Electronics, Hialeah, Fla.). We used the method of Brecher *et al*(4) as applied by Weed and Bowdler to determine the critical hemolytic volume of erythrocytes in hypotonic saline(5).

Red blood cells were diluted 1:25,000, resulting in a final concentration of approximately 200,000 cells per ml. Various concentrations of phosphate buffered sodium chloride solutions (pH 7.4) were used and the cellular volumes were expressed as the fraction V/V^o, in which V^o equalled the volume of the red blood cell in a 1.0 percent buffered sodium chloride solution. Previous studies have demonstrated that alterations in the ionic concentration of the conducting medium do not alter measurements of the particle volume(6). Likewise, Weed and Bowdler showed that the volume of red blood cells in 1.0% saline solutions was similar to cells suspended in filtered plasma(6). The reason red blood cells require hyperosmotic concentrations of sodium chloride to maintain their volume is obscure(4,6). Since only relative changes in cellular volumes were important for interpretation of our results, explanations for this phenomenon are irrelevant to these experiments. Cellular suspensions were maintained in test solutions for 45 minutes before sizing. Preliminary experiments showed that

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