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RICKETTSIOSES (TICK TYPHUS, Q-FEVER, URBAN TYPHUS) IN MALAYA[1]

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Abstract: Tick-borne rickettsiae occur primarily in the climatic climax forest formations which still cover much of peninsular Malaya. The jungle rats (*Rattus bowersi, R. canus, R. cremoriventer, R. edwardsi, R. rajah, R. sabanus, R. surifer, R. muelleri,* and *R. whiteheadi*) with their ectoparasitic *Dermacentor, Ixodes* and *Haemaphysalis* ticks are the reservoirs of tick typhus and Q-fever rickettsiae.

The basic Q-fever cycle was determined to involve primarily the jungle rat, *R. sabanus,* from which a strain of *Coxiella burneti* was isolated. Serological evidence of Q-fever infection in *R. surifer, R. muelleri* and *Tupaia glis* also implicated these forest animals in the basic Q-fever cycle. The presence of Q-fever complement fixing antibody in a house rat, *R. r. diardi,* in a north Malaya village indicates that domiciliated animals may also become involved. There is little evidence that domestic animals support a secondary cycle independent of the cycle in wild animals although cattle may occasionally become infected. Human Q-fever too is closely related to jungle exposure. All six of the serologically positive humans detected among the 216 sampled were soldiers who had spent time in the forest and, presumably, became exposed to a forest cycle of Q-fever.

The basic cycle of tick typhus also occurs in the mammals and ticks of climatic climax forests. The giant and spiny-furred forest rats (*R. rajah, R. surifer, R. sabanus, R. muelleri, R. edwardsi*), tree shrews (*Tupaia glis*) and *Ixodes granulatus* and *Haemaphysalis* spp. ticks are involved in the cycle. Presumptive isolations of tick typhus rickettsiae were made from *R. muelleri* and *R. whiteheadi* and from *I. granulatus* and *Haemaphysalis* spp. in climax dipterocarp forests. The other forest species mentioned were implicated on serological grounds.

Presumptive isolation of tick typhus rickettsiae from *R. r. argentiventer* and *R. r. diardi* collected in agricultural land, and the high prevalence of serological positives in urban rats indicate that a cycle also exists among domiciliated animals. This cycle, however, is probably secondary to the forest cycle with the animals living in man-dominated habitats receiving their infection indirectly from forest rodents. It was demonstrated that forest rats, particularly *R. bowersi, R. muelleri* and *R. whiteheadi,* wander into secondary forest-scrub ecosystems where they may come into contact with semi-domiciliated rodents (*R. r. jalorensis* and *R. exulans*) and exchange ectoparasites and rickettsiae. These in turn are in contact with domiciliated mammals (*Mus musculus, R. r. argentiventer, R. r. diardi* and *Suncus murinus*) in strictly man-dominated ecosystems—agricultural land, estates and urban areas. There is also serological evidence that scrub inhabiting birds may become infected with tick typhus, thus forming another potential link between forest and domiciliated animals through which rickettsiae may spread.

The edaphic climax formations of freshwater swamp and mangrove swamp forests were found to play a minor role in the ecology of tick-borne rickettsiae. The limited fauna of the mangrove swamp forests is generally isolated from climatic climax forests by wide tracts of intensively worked agricultural land and there is little chance of direct contact with the basic forest cycles. The freshwater swamp forests may be adjacent to primary dipterocarp forests, secondary forests and estates. Primary and secondary forest rats wander into the swamp forests as do rats from the estates. The low prevalence of serologically positive edaphic climax swamp forest animals indicates that tick-borne rickettsiae may be slowly transmitted into these ecosystems, but it is doubtful if independent cycles can be maintained there.

Urban or flea-borne typhus occurred only in the heavily man-dominated ecosystems (urban, and agricultural). The house rat, *R. r. diardi,* was most often implicated both serologically and by presumptive isolation of the organism. Other domiciliated or semi-domiciliated species, *R. exulans, R. r. jalorensis* and *Suncus murinus* also possessed either antibody or rickettsiae. The only evidence of urban typhus outside strictly man-dominated habitats was the finding of one sero-positive bird in a secondary forest-scrub habitat.

In a brief serological survey, psittacosis antibody was found in a high proportion of *Macaca irus* (25/132) and in 2/69 *Presbytis cristatus.* None of the 66 other wild mammals of several species tested had psittacosis complement-fixing antibodies.

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PART 1. GENERAL ECOLOGY

The most important rickettsiosis in Malaya in terms of human disease is scrub typhus. This disease has been known to exist in Malaya since the early days of the present century when Fletcher & Field (1927) showed that the so-called tropical typhus was actually the same as river fever (tsutsugamushi fever) in Japan and Schüffner's disease in Indonesia. Considerable effort over the past half century has been devoted to the study of scrub and urban typhus, and rightly so. However, the other rickettsial diseases have been neglected until very recently when the Institute for Medical Research (IMR) and the U. S. Army Medical Research Unit based at the IMR began to take an interest in Q-fever and other tick-borne rickettsioses (IMR 1959, 1960). The results of this work left no doubt that both Q-fever and tick typhus are present in Malaya.

The tick-borne diseases are zoonoses and usually have an animal cycle completely independent of man. Wild rodents or other small mammals of the fields and forests and their ectoparasitic ticks are the most likely candidates for reservoir and maintenance hosts, the ticks also serving as vectors. The purpose of the present investigation was to study the tick-borne rickettsioses in the small mammals and ticks of Malaya using a broad ecological approach. An attempt was made to explore as many different habitats over as wide an area as possible, but for practical reasons, the work was concentrated in the vicinity of Kuala Lumpur and the western part of the Malay Peninsula.

Before discussing the rickettsioses themselves, however, it is necessary to outline in a general way the ecology of Malaya and in more detail that of the mammals and ticks. This section is thus devoted to describing the major ecosystems that may be important in the natural history of the rickettsial diseases under investigation.

METHODS

Wild animals were collected by live-trapping with the assistance of an experienced aborigine trapper. Collections were made from as many ecologically distinct areas as possible, but were primarily from areas in the states of Kedah, Penang, Perak, Selangor, Negri Sembilan, Johore and Malacca in the western part of the Malay Peninsula, and Pulau Aur, an island off the southeast coast (Table 1, Fig. 1). An effort was made to trap all habitats within each area so that all the species present would be collected. Most areas were trapped continuously for at least 4 weeks. In this way it was hoped that a crude measure of the relative number of each species in an area would be obtained. The success of this attempt varied from area to area depending upon the number of species populating a specific habitat and the relative ease with which they could be trapped. However, the experience of our aborigine trapper[3] and his knowledge of the fauna helped to assure that adequately representative samples of the ground fauna were obtained from most areas. No effort was made to get a representative sample of the canopy-dwelling species because of the extreme difficulty in trapping these animals.

Live traps made from 1.9 cm wire mesh were used routinely. Similar traps of 0.6 cm wire mesh were used to collect house mice in Kuala Lumpur. As a general practice, traps in the forest were baited with bananas, dried coconut or tapioca root. Occasionally nangka and other fruits were used. During the durian season, these fruits were the preferred bait for forest animals. By varying the bait to suit the area, it was hoped that the maximum number and variety of animals

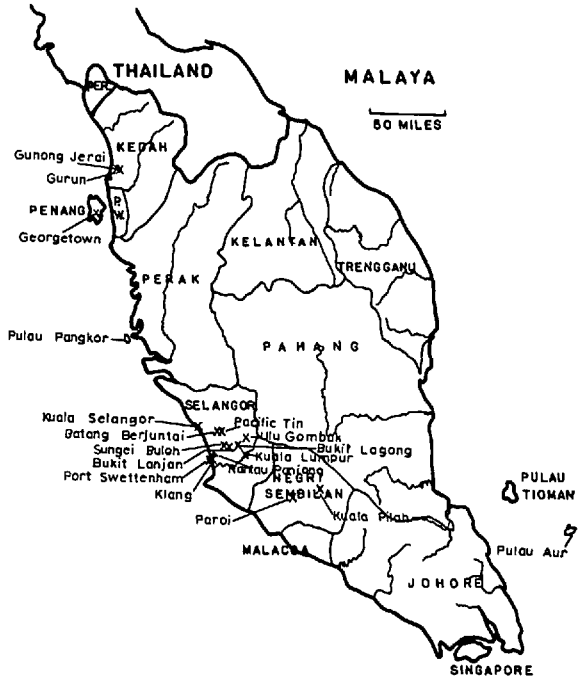

Fig. 1. Small mammal collecting sites in Malaya.

3. The same trapper, Sipang, was used throughout the 2-year study. He is an extremely knowledgeable person with respect to the forest fauna and flora; and has had over 13 years of trapping experience working for various investigators at the IMR.

Table 1. Small mammal sampling areas in Malaya.

Habitat	Collecting Area	State
Mountain Oak Forest	Gunong Jerai	Kedah
Upper Dipterocarp Forest	Gunong Jerai	Kedah
Hill Dipterocarp Forest	Bukit Lagong* and Ulu Gombak* Forest Reserves	Selangor
Lowland Dipterocarp Forest	Bukit Lanjan* and Kuang Forest Reserve	Selangor
Secondary Forest Scrub	Subang*, Bukit Lagong Lowlands & Bukit Lanjan*	Selangor
Freshwater Swamp Forest	Pacific Tin and Batung Berjuntai	Selangor
Mangrove Swamp Forest	Rantau Panjang*	Selangor
Islands	Pulau Aur	Johore
	Pulau Pangkor	Perak
Padi Agricultural Land	Malacca	Malacca
	Paroi	Negri Sembilan
	Kuala Pilah	Negri Sembilan
	Gurun	Kedah
	Gunong Jerai	Kedah
Estates	Sungei Buloh, Rantau Panjang	Selangor
Urban Areas	Kuala Lumpur*, Klang	Selangor
	Georgetown	Penang

* Trapping areas which have been intensively studied for several years by the Institute for Medical Research.

would be obtained.

The trapping areas are listed in Table 1 and shown on the accompanying map (Fig. 1). Some of them have been studied intensively for many years (Audy & Harrison 1954; Gordon-Smith *et al* 1961), while others have been studied poorly or not at all.

RESULTS AND DISCUSSION

Malaya is still approximately 70% virgin tropical rain forest, but the eastern and particularly the western coastal plains are under intensive agricultural development. Between the coastal plains and the virgin rain forests is a ragged belt of man-disturbed land, now mostly *belukar* (secondary forest) and scrub—the habitat of scrub typhus (Audy & Harrison 1951). Within these large biomes, distinct ecosystems can be delineated, the number depending upon the criteria used. From a botanical point of view, the Malayan vegetation can be divided into 17 main types or ecosystems (Symington 1943), but, useful as this division may be to plant ecologists, a broader classification with fewer types more appropriately satisfied our purposes. Thus, on the basis of dominant plant formations and degree of human disturbance, 11 broad ecological divisions can be recognized. Each of these habitats has a distinct faunal composition, although in the case of the smaller mammals, there may be considerable overlapping in some species which have wide habitat tolerances. Some, however, are quite specific in their habitat and can often be used as indicators of certain ecosystems.

Among the 11 ecosystems sampled were four of the five climatic climax formations of Symington (1943): Mountain-Oak Forest, Upper Dipterocarp Forest, Hill Dipterocarp Forest and Lowland Dipterocarp Forest (Table 2). The Montane-Ericaceous Forest formation of the high mountain peaks over 1500 m elevation was not studied. Only the tallest mountains in Malaya rise above 1500 m and are able to support this formation which is composed chiefly of stunted trees 3–6 m high and of mosses, lichens and epiphytic shrubs (Wyatt-Smith 1952 b). Two ground dwelling rats, *Rattus fulvescens* (Gray) and *R. alticola* (Thomas) generally not found elsewhere, occur here (Harrison & Traub 1950), and there is evidence that these high mountain rats may be involved in the ecology of tick typhus (IMR 1960) (see Part 2).

1. Mountain Oak (Montane) Forest

The sampling areas between 960 m and the summit at 1200 m on Gunong Jerai in Kedah are characteristic of mountain-oak forest. The flora of this zone on G. Jerai contains a considerable portion of genera of Australian origin: *Leptospermum flavescens*, *Tristania merguensis*, *Baeckia frutescens*, and the conifers, *Agathis alba*, *Dacrydium elatum*, *D. beccarii* and *Podocarpus neriifolius*. The other dominant group is the oak, *Quercus* spp.

The trees are short (6–12 m), and often clothed with moss, orchids and other epiphytes and parasites. There is no emergent layer of vegetation, and the ground, rocks and trees are covered with mosses, lichens, orchids and other herbaceous plants (Ridley 1916; Marchette 1965). Particularly noticeable dur-

ing the wet season in more or less open areas along roads, trails and wasteland is the purple-flowered *Burmannia disticha*.

The fauna consists of at least nine species of small ground mammals (plus several bats), carnivores (civets and cats), monkeys, gibbons and the larger ungulates (tapir, wild pig, and deer). The most abundant small mammals in the trapping collection made in March

Table 2. Small wild mammals* of Malaya collected for study, and the habitats in which they were trapped.

Species	Mountain Oak Forest	Upper Dipterocarp Forest	Hill Dipterocarp Forest	Lowland Dipterocarp Forest	Secondary Forest Scrub	Freshwater Swamp Forest	Mangrove Swamp Forest	Plantations (Rubber, Oil Palm, Coconut)	Agricultural Land (Padi, Farms)	Urban Areas	Islands	Total
RODENTIA												
Bandicota indica (Bechstein), Greater bandicoot									1			1
Callosciurus caniceps (Gray), Grey bellied squirrel			3		3				1			7
C. hippurus (Geoffroy), Horse-tailed squirrel			1									1
C. nigrovitatus (Horsfield), Black-banded squirrel			13	5	1							19
C. notatus (Boddaert), Red bellied squirrel	16	1	30	11	2	71	20	10			2	163
C. prevosti (Desmarest), White-striped squirrel						3						3
C. tenuis (Horsfield), Slender little squirrel				2		3						5
Lariscus insignis (Cuvier), 13-striped ground squirrel				1								1
Rhinosciurus laticaudatus (Muller & Schlegel), Shrew-faced ground squirrel			4	1		4						9
Chiropodomys gliroides (Blyth), Bamboo tree mouse			4		1							5
Mus musculus L., House mouse										21		21
Rattus annandalei (Bonhote), Singapore rat					2	46						48
R. bowersi (Anderson), Grey giant rat	5		23	9	21							58
R. canus (Kloss), Grey tree rat			8									8
R. cremoriventer (Miller), Dark-tailed tree rat	10		8	2	1							21
R. edwardsi (Thomas), Mountain giant rat	4	1										5
R. exulans (Peale), Burmese rat					8	2	1	1	20	9	3	44
R. muelleri (Jentink), Swamp giant rat			75	12	21							108
R. norvegicus (Berkenhout), Norway rat										41		41
R. rajah pellax (Miller), Rajah spiny rat	14	8	13	16	2							53
R. rattus argentiventer (Robinson & Kloss), Ricefield rat									29	3		32
R. r. diardi (Jentink), Malaysian house rat							1	33	69	140		243
R. r. jalorensis (Bonhote), Malaysian woodrat					26		45	98	34		2	205
R. r. roa (Miller), Malaysian house rat											68	68
R. sabanus (Thomas), Long-tailed giant rat			70	33	5							108
R. surifer (Miller),** Red spiny rat	11	4	4								72	91
R. whiteheadi (Thomas), Little spiny rat	27	3	7	18	25	12						92
INSECTIVORA												
Echinosorex gymnurus (Raffles), Moon rat			8	3	1	2	1					15
Hylomys suillus (Müller), Short-tailed shrew	1		2									3
Suncus murinus (L.), House shrew									5	95		100
PRIMATES												
Tupaia glis (Diard), Common tree shrew	33	6	13	19		1			1		31	104
T. minor Günther, Lesser tree shrew			6	2								8
Total	121	23	292	134	119	144	68	142	160	309	178	1690

* The species names are based on Chasen (1940) and Ellerman & Morrison-Scott (1955).

** Includes *R. surifer aoris* (Robinson) from Pulau Aur and *R. surifer surifer* (Miller) from the mainland.

and August, 1963 were, in their order of abundance, *Tupaia glis* (tree shrew), *Rattus whiteheadi*, *Callosciurus notatus*, *R. rajah*, *R. surifer*[4] and *R. cremoriventor* (a tree rat) (Table 2). A few *R. bowersi* and *R. edwardsi* and the short-tailed shrew, *Hylomys suillus*, complete the main faunal components of this habitat as determined by live trapping methods. Domrow & Nadchatram (1963) had essentially the same results when they trapped this area earlier.

The water rat, *R. muelleri*, was not represented in the collection since its preferred habitat along sloping stream banks is lacking. There is a small stream on the mountain, but it is quite short, plunging eventually down the NE-facing cliffs and is not formed by sloping banks. The moon rat, *Echinosorex gymnurus*, was also missing and probably for the same reason. It too lives by forest streams and feeds on fish and other aquatic animals (Harrison 1957b).

At about the 960 m level on G. Jerai there is a hill station and beyond it are several houses where road and telecommunications maintenance workers live. Near the living area is a well-tended garden for the table of the Sultan of Kedah. Between the houses, gardens, and roads is a wasteland of grass and weeds. This island of human disturbance surrounded by mountain-oak forest contains a much different fauna, being composed of the man-associated species, *R. r. argentiventer* and *Bandicota indica*, typically rodents of the padi fields; and the Malayan house rat, *R. r. diardi* which lives in and around the houses. These animals are not forest species and are not part of the mountain oak forest fauna. In Table 2 they have been listed under the appropriate habitat.

2. Upper Dipterocarp[5] Forest

In this forest zone which extends from about 750 m to 960 m on Gunong Jerai, *Leptospermum flavescens*, *Tristania merguensis* and the conifers are absent. The area is less xerophytic than the mountain-oak forest above it, and the understory tends to be more developed. Mosses, orchids and other epiphytes are still a prominent feature of the flora, but saprophytic herbs are more abundant. Only a few species of the Dipterocarpaceae occur here in contrast to the vast number characteristic of the dipterocarp forests of lower elevations.

The species composition of this ecosystem was essentially the same as in the mountain-oak forest with *T. glis* a predominant part of the small mammal population. *Rattus cremoriventer* was missing, but the sample collected was too small to draw any significance from the apparent absence of this tree rat. It is not often trapped, but was taken in hill-dipterocarp forests at lower elevations (see also Harrison & Traub 1950).

3. Hill Dipterocarp Forest

The richest forest habitat is the hill-dipterocarp zone lying usually between 300 and 750 m elevation on the main inland range of mountains and at lower elevations on coastal hills. The areas sampled in this study are on the west slope of the main range in Selangor near Kuala Lumpur. Species of Dipterocarpaceae are prominent, forming the emergent layer of vegetation with other large trees 30–45 m tall. In the understory are gingers, many species of Rubiaceae, figs and a few palms. The floor is open except where breaks in the canopy let through enough light to promote heavy growth of seedlings, shrubs and stands of bamboo. The tremendous variety of orchids seen in the higher formations is here reduced to only a few terrestrial varieties. Gingers and members of the family Araceae are commonly encountered on the forest floor. Many species of shrubs and herbaceous plants are scattered about, growing wherever there is sufficient light.

The animal fauna is equally rich with at least 18 species of small ground-dwelling mammals, as well as several species of high tree dwellers such as the squirrels, *Ratufa bicolor* (Sparrman) and *R. affinis* (Raffles). The dominant rodents in the areas sampled were *R. sabanus* and *R. muelleri*, the latter being always associated with streams. These two species composed approximately 50% of the catch of nearly 300 mammals made in several areas of hill forest. Next in order of abundance were the common squirrel, *C. notatus*, the wandering ground rat, *R. bowersi*, the tree squirrel, C. *nigrovitatus*, the tree shrew, *Tupaia glis*, the tree rat, *R. canus*, and the ground-dwelling *R. rajah*. Rudd (1965) collected both *R. rajah* and *R. surifer* from this ecosystem at Ulu Gombak, but found them occupying different habitats on the forest floor. Other species were taken only occasionally (Table 2), some from specialized habitats within the forest, e.g.,

4. Ellerman & Morrison-Scott (1951) list these species as subspecies of *R. rajah* (*R. rajah pellax* and *R. rajah surifer* respectively). However, Harrison (1962) separates them into 2 species *R. rajah pellax* (Miller) and *R. surifer* (Miller) on the basis of fur and hair color, thickness of tail base, and size of tail scales. Lim (1965) concurs with this view and considers them to be ecologically as well as morphologically distinct. *R. surifer* tends to be confined to higher elevations preferring dry places in the forest and is very widespread in Southeast Asia. *R. rajah* occurs in moist or marshy habitats in the lowlands as well as in the higher elevations and has a more restricted distribution.

5. The term Dipterocarp refers to trees of the Meranti family or Dipterocarpaceae which form a dominant part of the emergent layer of this and the next three forest habitats.

Chiropodomys gliroides in bamboo thickets where it nests in the hollow bamboo internodes. The moon rat, *Echinosorex gymnurus*, was fairly common in this habitat and in the lowland dipterocarp forests but is never abundant. *Rattus cremoriventer* was fairly abundant and *R. whiteheadi* was common to all forest formations.

4. Lowland-Dipterocarp Forest

This formation often borders man-disturbed areas of cut-over or burned-over land and secondary forest. The lowland-dipterocarp forests sampled are in Selangor near Kuala Lumpur. The Dipterocarpaceae, forming about 50% of the emergent layer, are greatly exploited for their lumber, and logging operations have disturbed much of the lowland forests of Malaya, particularly along the west coast. There are, however, still vast stretches of virgin or essentially virgin stands of timber throughout the country. The forest is dense and contains many thousands of species in four, more or less distinct, layers: the emergent layer with trees to 45 m tall, the main story with trees to 30 m tall, an understory of small trees and saplings, and the basal layers of shrubs, seedlings and herbs. The shrub layer contains many saplings of the trees in the higher stories as well as species of Euphorbiaceae, Rubiaceae and Annonaceae. In some areas palms are a prominent component of the flora. This is a dense layer, but it is often quite open at ground level, particularly where the canopy overhead has remained intact. The herb layer consists of seedlings of the trees and shrubs above, and ferns and herbaceous plants of many genera scattered throughout the forest. Seldom does any one species occur in great numbers or in pure stands.

This is also an area rich in species of small mammals with at least 14 ground dwelling forms commonly found. About 25% of all the animals trapped in this formation were *R. sabanus*, the same proportion as in the hill-dipterocarp formation. Locally along streams, *R. muelleri* again was common. *Tupaia glis*, *R. rajah*, *R. whiteheadi*, *R. bowersi* and *C. notatus* complete the list of the most common ground dwelling species. The moon rat, *E. gymnurus*, was occasionally trapped as were *R. cremoriventer* and *C. nigrovitatus*. More of the latter two tree dwelling species and perhaps *R. canus* might have been collected if more intensive trapping in the trees had been done. No *R. surifer* were collected but *R. rajah* was obtained in some abundance.

5. Secondary Forest Scrub

This is not a climax formation, but a transitionary development in the plant succession from cut-over or burned-over climatic climax forest (usually lowland-dipterocarp) back to climax forest. In all areas studied, man has been the disturbing factor. There are two rather distinct formations in this division—secondary forest (*belukar*) and lalang or grass-wasteland. However, they are included in one ecosystem here on the basis of disturbance by man rather than on the basis of floristic formations. *Belukar* and lalang usually are found adjacent and, to a large extent share the same small mammal species.

The secondary forest is a dense tangle of vegetation containing few or no trees of great size, particularly in the early stages of regeneration. When the emergent and main story layers of the forest are cut and/or burned, sunlight in abundance reaches the shrubs and herbs stimulating them to rapid development until a thick mass of vegetation results. The flora of old secondary forest is dominated by species of Euphorbiaceae and Urticaceae and by Myrtaceae, Rhizophoraceae and Lauraceae. The Rubiaceae are of much less importance here than in climatic climax forest and the Dipterocarpaceae are entirely absent. In the early stages of secondary forest formation, some of the most conspicuous plants are *Eupatorium*, *Ageratum*, *Lantana* and *Melastoma malabathricum*.

Lalang is a biotic climax formation (Wyatt-Smith 1952a) and is a result of repeated burning of fields. The hardy grass, *Imperata cylindrica*, with its subsurface rhizomes survives the fires and springs up anew as soon as man's cultivation attempts abate. This tough grass, nearly unpalatable to herbivores, may reach 1.2–1.8 m and provides excellent cover for the many small animals, although the mammal fauna is poor in species.

The most abundant small mammals are two man-associated rats (*R. r. jalorensis*[6] and *R. exulans*) and three forest rats (*R. bowersi*, *R. muelleri* and *R. whiteheadi*). *Rattus r. jalorensis* is never found in undisturbed primary forest, but is at home wherever man has made clearings or other disturbances. *Rattus exulans*, the other man-associated rodent, seldom occurs in high density, but is widespread throughout Malaya in houses, wasteland, lalang, gardens and padi.

Of the three forest rats, *R. whiteheadi* is equally at home in secondary forest, wasteland and primary forest. *Rattus bowersi*, although thought to occur only in the forest (Harrison & Traub 1950), comprised 18% (28/119) of the animals collected from the secondary forest-scrub formations trapped. Most of them came from the secondary forest rather than the lalang-wasteland areas. They are noted for their wide range and many may well have originated in adjacent forest

6. Dhaliwal (1961) considers *jalorensis* and *diardi* to be distinct subspecies of *Rattus rattus* on the basis of the ecological preferences of the two animals.

areas. The relatively large number of *R. muelleri* (18%) collected reflects the presence of streams in the collecting areas.

Occasionally true forest species (*R. sabanus*, *R. cremoriventer*, *R. rajah*, etc.) were taken, but they undoubtedly were animals that strayed from the nearby forest and do not constitute part of the permanent fauna dwelling in secondary forest.

6. Freshwater Swamp Forest

The areas classified as freshwater swamp forest include the semi-swamp (lopak) forests of Symington (1943) and true swamp-peat forest. They are edaphic climax formations located on flat land which is periodically, but not permanently, inundated. The areas studied are located in north Selangor state. Characteristic plants, to name only a few, are palms and screw pines (*Pandanus* spp.), *Eugenia* spp. and the squat pitcher plant, *Nepenthes ampullaria*. It is a difficult area in which to work because of the dense vegetation in places where sunlight penetrates, and the thick mud soil.

The small mammal fauna, poor in species, is dominated by *C. notatus* (48%) and *R. annandalei* (32%). The latter is closely related to *R. muelleri* which, however, prefers a stream bank habitat in climatic climax and secondary forests. On Singapore Island, where it is called the Singapore rat, *R. annandalei* occurs in secondary forest and scrub (Dhaliwal 1961; Harrison 1962). Elsewhere it is found along the edge of forest formations (Audy & Harrison 1954), and may extend for some distance into hill dipterocarp forest (see Part 2; Rudd 1965). *Rattus whiteheadi* again occurred in relatively large numbers and constituted 8% of the collection. A few squirrels, *C. prevosti*, *C. tenuis* and *Rhinosciurus laticaudatus*, were collected from the lopak or semi-swamp portion of this ecosystem. *Callosciurus prevosti* invades this " edge " habitat when it borders on plantations; the other two squirrels when the adjacent habitat is lowland dipterocarp forest.

7. Mangrove Swamp Forest

The saline mud of the mangrove swamp supports only a few plant species. The most conspicuous are the *Rhizophora* with their stilt roots, the *Avicenna* and *Soneratia* with their pneumatophores sticking out of the mud, nipa palms (*Nipa fruticans*) and nibong palms (*Oncosperma tigillarium*). The aerial roots, pneumatophores, and the mud make crossing a mangrove swamp extremely difficult and unpleasant. For an excellent general description of Malayan mangrove forests see Wyatt-Smith (1953a), and for a detailed description see Watson (1928).

The mangrove swamp forest at Rantau Panjang near Klang in Selangor, where the collecting was done, borders on a small coconut plantation. There was undoubtedly some exchange between swamp dwelling mammals and those in the coconut plantation.

The animal fauna is limited, the most common species being the mud-skipper (*Periophthalmus*), mud lobster (*Thalassina anomala*) and mangrove crabs (*Uca* spp. and *Sesarma* spp.), as well as monitor lizards and crab-eating monkeys (*Macaca irus*). The small-mammal fauna is essentially restricted to two species, *R. r. jalorensis* and *C. notatus*, comprising 66% and 29% of the collection respectively. These species are abundant in the mangrove swamp near the landward edge, but probably diminish in number the further one penetrates towards the tidal marshes.

8. Islands

This is not, properly speaking, a specific ecological division, but rather a combination of habitats surrounded by water and isolated from contact with other land areas. The species composition of islands may be determined more by circumstances and accidents than by ecological preferences of the species so that introduced species tend to inhabit whatever habitat there is. As an illustration, on Pulau Aur, where most of the island specimens were collected, nearly half the animals were *R. surifer aoris*—all from the lowland-dipterocarp forest. However, *Rattus rattus roa* also occurred in the forest, although its main habitat preference tended to be in the kampongs. *Rattus rattus* never occurs in forests on the mainland. The only other species trapped in the forest was *Tupaia glis*, primarily a forest species, but here it was also found in the coconut plantations and *belukar* near the kampongs. In and around the kampongs, *R. r. roa* was extremely abundant, but no *Suncus murinus* (house shrews) were trapped. An occasional *C. notatus* was taken from the coconut plantations.

On Pulau Pangkor, much closer to the mainland than P. Aur, the dominant animals in the man-disturbed areas and villages were *R. r. diardi* and *R. r. jalorensis* as on the mainland. The latter species was missing from P. Aur. In the Simbilan Islands off the coast of Perak and on the island of Berhala off the coast of Sumatra, a form of *R. r. jalorensis* (*R. r. rumpia* Robinson & Kloss), the only rat found, occupies forest as well as other habitats (Harrison 1957a). On Pulau Jarak the only rat known is *R. r. diardi* (called *R. r. jarak* (Bonhote)), a house rat on the mainland (Audy *et al* 1950; Wyatt-Smith 1953b).

9. Plantations

The large estates of Malaya are primarily devoted to the cultivation of rubber, oil palm and coconut. These are generally well tended with the trees planted

in neat rows or at least widely spaced. The understory vegetation is kept cleared and often certain plants are cultivated between the tree rows as green manure. There is considerable daily disturbance by man, and the fauna reflects this situation. The most abundant small mammal in the plantations of the Malayan west coast was *R. r. jalorensis* which constituted 69% (98/142) of the catch. *Rattus r. diardi*, the house rat, constituted 23% and *C. notatus*, 7%. An occasional *R. exulans* also occurred in this habitat. The tree squirrels, *Callosciurus prevosti* and *C. erythraeus*, are common pests in oil palm estates (Harrison & Traub 1950), but none were collected.

10. Agricultural Land (padi and truck gardens)

Most of the collections in this habitat were made in padi fields and vegetable gardens, the latter always in close proximity to human habitation. The most abundant small mammals were *R. r. diardi* and *R. r. jalorensis*. The padi field rat, *R. r. argentiventer*, abounded in the padi fields but was rarely found elsewhere except in nearby gardens. *Rattus exulans* was also fairly common. Only rarely are other species collected in this habitat, except for *Bandicota* spp. in the padi fields of northern Malaya.

11. Urban Environment

This ecosystem in which man is the dominant animal, includes farm houses, small villages, suburban developments and cities. Animals were collected from inside as well as around houses, largely in Kuala Lumpur, but also from coastal cities and villages. The house rat, *R. r. diardi*, was the most numerous small mammal with 45% (140/309) of the catch. The house shrew, *Suncus murinus*, found only in close association with man, was second in number (31%). The house mouse, *Mus musculus*, was less abundant, but these small creatures were taken only in special traps with small mesh wire screen, and the number captured may only reflect the effort made to trap them. The Norway rat, *R. norvegicus*, was taken only in coastal cities. *Rattus exulans* is particularly common in squatters huts outside of town (Harrison 1949) where it is second in importance to *R. r. diardi* as a house rat (Harrison 1962). In this study it was only occasionally captured in the urban areas sampled.

In Kuantan to the east coast and Kuala Tahan in

Table 3. Ticks collected from small mammals in Malaya.*

Mammal Hosts		*Derm. auratus*	*Ix. granulatus*	*Haemaphysalis*	*Amblyomma*	Total	Average No. of ticks/animal
Species	Number	Supino	Supino	spp.**	spp.		
Bandicota indica	1	1	3			4	4
Callosciurus notatus	155	27		22		49	0.32
C. tenuis	4	7				7	0.57
Echinosorex gymnurus	11	6	15			21	2.0
Rattus annandalei	36	4	43	1		48	1.33
R. bowersi	58	141	3	75	1	220	3.80
R. cremoriventer	20		2	2		4	0.20
R. edwardsi	5	40	5	9		54	10.80
R. exulans	43		1			1	0.02
R. r. argentiventer	32	2	2			4	0.12
R. r. jalorensis	190	4	6	1	2	13	0.07
R. r. roa	65		89			89	1.37
R. muelleri	105	43	31	3		77	0.73
R. sabanus	95	93	34	17		144	1.52
R. rajah	52	34	8	6		48	0.92
R. surifer	83	15	29	15		59	0.77
R. whiteheadi	82	6	5			11	0.13
Rhinosciurus laticaudatus	5		13	1		14	2.8
Tupaia glis	88	8	32	6	3	49	0.56
T. minor	4	1				1	0.25
Total	1134	432	321	158	6	917	0.81

* In addition to those listed, 12 *Rhipicephalus sanguineus* Supino were collected from 2 dogs, 2 *Amblyomma testudinarium* Koch were found crawling on humans, 84 *Haemaphysalis centropi* Kohls were taken from several species of ground birds, 73 *Amblyomma javenense* (Supino) were collected from 1 *Manis javanica*, 1 *Dermacentor auratus* Supino and 1 *Haemaphysalis* sp. were taken from 1 *Sus scrofa*, 57 *Amblyomma helvolum* Koch were collected from 2 *Varanus salvator* (Lauri) and 27 *Heamaphysalis bispinosus* Neumann were collected from a white rabbit staked out in a cattle field.

** Probably *H. papuana* Thorell and *H. semernis* Neumann according to Nadchatram (1965).

central Pahang, where *R. r. diardi* does not occur, *R. r. jalorensis* has become adapted to houses (Harrison 1957a).

The ticks from mammals collected throughout Malaya are tabulated in Table 3. *Dermacentor auratus*, *Ixodes granulatus* and *Haemaphysalis* spp. were the predominant ixodid ectoparasites of the small wild mammals trapped. Occasionally *Amblyomma* spp. were found, but their prevalence was very low except on certain animals such as the scaly anteater, *Manis javanica* Demarest, which is heavily parasitized by the host-specific *A. javanensis*. The only other mammals on which *Amblyomma* ticks were collected were *R. bowersi*, *R. r. jalorensis* and *Tupaia glis*.

The forest species of rodents are the major hosts of the larval and nymphal stages of *D. auratus*, and it is the commonest immature tick parasitizing rodents in Malaya. *Rattus bowersi*, *R. edwardsi*, *R. muelleri*, *R. sabanus*, *R. rajah* and *Rh. laticaudatus* had the highest number per individual (Table 3). Only six of the 155 *C. notatus* trapped had *D. auratus* on them; three were from mountain-oak and three from fresh-water swamp forests. Almost half of the ticks collected from small mammals were *D. auratus*. The adults in Malaya primarily parasitize wild pig (*Sus scrofa* L.)

Ixodes granulatus is the only tick listed by Audy *et al* (1960) as infesting the moon rat, *E. gymnurus*, but in the present collection, two specimens trapped in lowland dipterocarp forest had attached to them 2 and 4 *D. auratus* respectively.

With the exception of *R. annandalei*, the major hosts of *Ixodes granulatus* were mountain-dipterocarp forest species of small mammals. *Rattus annandalei* had a high index of 1.33 ticks per animal. *Tupaia glis* was host to more *I. granulatus* than to any other tick and had an index for this species of about 0.36. According to Audy *et al* (1960) the giant forest rats, *R. muelleri* and *R. sabanus* are the major hosts; the current study tends to bear this out. Although parasitic on rodents in all stages, almost every *I. granulatus* collected was an adult.

The adult *Haemaphysalis* spp. are parasites of the carnivora and the immature forms parasitize giant forest rats (Audy *et al* 1960). In this study the major hosts of immature *Haemaphysalis* ticks were *R. bowersi* and *R. edwardsi*. Other animals were infested with them but at very low levels (Table 3).

In terms of overall tick infestation the climax dipterocarp forest species, *R. bowersi*, *R. edwardsi*, *R. muelleri*, *R. sabanus*, *R. rajah*, *R. surifer*, *Rh. laticaudatus* and *T. glis* had the highest tick indices. The swamp forest rat, *R. annandalei*, and the moon rat, *E. gymnurus*, which occurs in several ecosystems wherever there are streams, also had high indices. Man-associated animals had very low rates of infestation. The wide ranging *C. notatus* contained relatively few ticks (Index of 0.32) and no *I. granulatus*, but Kohls (1957) lists it as a parasite of this squirrel.

Few ticks were collected from the man-associated rodents, *R. argentiventer*, *R. exulans*, and *R. r. jalorensis*. *Rattus r. jalorensis*, however, *R. bowersi* and *T. glis* were the only animals from which ticks of all four genera were collected. *Rattus muelleri*, *R. rajah* and *R. sabanus* also are known to be host to all these ticks (Audy *et al* 1960), but no *Amblyomma* were collected from them in this study. Their diverse tick fauna reflects the wide range of habitats frequented by these species, although *R. whiteheadi*, with an even larger habitat range, possessed only *Dermacentor* and *Ixodes* ticks.

No ticks were collected from mainland *R. r. diardi*, but *I. granulatus* was taken from *R. r. roa* trapped on Pulau Aur. *Ixodes granulatus* is apparently the only tick on the island. On Pulau Jarak, *R. r. jarak* was parasitized by *Dermacentor* and *Haemaphysalis*, but not by *Ixodes* (Audy *et al* 1960). The island habitat is populated by chance more than by design, and the early inhabitants tend to become the dominant species to the exclusion of other forms. It is possible that on P. Aur, no other ticks have been introduced—at least *I. granulatus*, probably introduced with *R. surifer*, was the only tick collected.

Analysis of the trapping results (Tables 2 and 3) indicates that a tick-borne microbial parasite could easily circulate through most or all the major ecosystems as defined in this study. This essentially includes all of Malaya. Whether they do or not is the subject of the following two sections. At least the opportunity for spread throughout the small mammal population and transmission to man and domestic animals exists. Possibly an extremely important habitat in this connection is the secondary forest-scrub formation where the climatic climax forest species (*R. bowersi*, *R. muelleri*, *R. whiteheadi*, and other species to a lesser extent) come into contact with man-associated rodents, particularly *R. r. jalorensis* and *R. exulans*. Here an exchange of ticks may readily take place and a rickettsia or other tick-borne parasite pass from forest rat to man-associated rat.

A most important tick in this scheme should be *D. auratus*, which parasitizes rodents in the larval and nymphal stages and changes hosts between stages. It is abundant on forest rats and not infrequently on *R. r. jalorensis* inhabiting secondary forest.

The same case can be made for *Haemaphysalis* ticks. The case for *I. granulatus* also seems clear-cut. It parasitizes small rodents in all stages, but for some reason, most of the *Ixodes* collected from small mammals were adults. Less than 1% of 321 specimens

were immature ticks in this collection, and Audy *et al* (1960) reported only 6% (38/593) larvae and nymphs from their collections. These were mostly on the giant forest rats, but *E. gymnurus* also possessed larval ticks. Immature *I. granulatus* feed for shorter periods of time than adults, and they attach singly to all parts of the body making collecting more difficult than for adults. These and other factors may be responsible for the apparent discrepancy between number of adults and immature ticks obtained from wild rodents. *Ixodes granulatus*, like other ixodid ticks, tends to feed only once during each phase of the life cycle and normally the adult will feed on a single host only, then drop off to lay eggs. It may, however, change hosts under certain circumstances, e.g., if its first host dies or is killed before it has had a full blood meal.

None of the small wild mammal ticks are serious parasites of man or his domestic animals, although there are records of tick bites or recoveries of all these ticks from humans. It would appear, therefore, that the chance for human involvement in tick-borne rickettsial disease in Malaya is slight; this likelihood will be discussed further in Parts 2 and 3.

SUMMARY AND CONCLUSIONS

The small mammals and ticks in the major ecosystems in Malaya were studied as a basis for studying tick-borne rickettsioses. These ecosystems can be grouped into four major divisions with the climatic climax forest formations (Mountain-Oak, Upper Dipterocarp, Hill Dipterocarp and Lowland Dipterocarp forests) of great potential importance to the ecology of tick-borne rickettsiae. In these vast areas, isolated from all but minor human disturbances, the jungle rats (*R. bowersi*, *R. canus*, *R. cremoriventer*, *R. edwardsi*, *R. rajah*, *R. sabanus*, *R. surifer*[7] and *R. whiteheadi*), and other small mammals with their ectoparasitic *Dermacentor*, *Ixodes*, *Haemaphysalis*, and, to a less extent, *Amblyomma* ticks form a potential reservoir for rickettsiae. Here the basic jungle cycles of Q-fever and tick-typhus exist, as will be shown in Parts 2–4. Two of these rats, *R. bowersi* and *R. whiteheadi* frequently wander into secondary forest and other ecosystems.

The secondary forest-scrub ecosystem constitutes a fringe habitat or an edge (Audy 1958) where forest mammals and their ectoparasites come into contact with semi-domiciliated rodents (*R. r. jalorensis* and *R. exulans*) and probably exchange ectoparasites and rickettsial infection. This fauna is in turn in contact with domiciliated mammals (*M. musculus*, *R. r. argentiventer*, *R. r. diardi* and *S. murinus*) in strictly man-dominated ecosystems—agricultural land, estates and urban areas. A potential link between man and the basic rickettsial cycles in forest mammals does therefore exist.

The edaphic climax formations of freshwater swamp forests and mangrove swamp forests probably play a minor role in tick-borne rickettsial ecology. The mangrove swamps of the coast contain a very limited small mammal fauna generally isolated from the climatic climax forests of the interior by wide tracts of intensively worked agricultural land.

The fresh water swamp forests may be adjacent to primary dipterocarp forests on one side and secondary forests or estates on the other. Jungle species may wander into them from one side and secondary forest or estate species from the other. Thus, this ecosystem may form a buffer between climatic climax forest and fringe habitat through which rickettsiae may be slowly transmitted.

PART 2. TICK TYPHUS[8]

Recent reports of serological evidence of tick typhus infection in wild rodents and humans in Malaya (IMR 1955, 1959, 1960) stimulated the present investigation of tick typhus ecology. There were no reports of this infection in SE Asia before the early 1950's (Wilcocks 1944 a–d), probably because no investigations were made. Serological evidence of human tick typhus, however, has since been obtained in Viet Nam (Cluzel & Roux 1953; Capponi 1956) where sporadic cases diagnosed as " fievre exanthematique benigne " were considered to be similar in etiology to boutonneuse fever (Cluzel & Desnues 1955; Nguyen-van-ai 1962; Beytout 1964). Evidence of tick typhus in Thailand has been accumulating and a rickettsial agent was recently isolated from ticks (Elisberg 1964). There are no reports of its presence in Indonesia, Cambodia, Laos or the Philippines, but it is known to exist in adjacent areas and probably occurs in these countries as well. The known distribution

7. A relatively large number of *R. surifer* (31) are listed under the insular habitat in Table 2, but these were actually collected in climax lowland dipterocarp forest on Pulau Aur and may be considered forest species. *Rattus surifer* appears to inhabit most of the small islands near the Malay peninsula (Chasen 1940).
8. This study was limited to the Malay Peninsula and some nearby islands extending from approximately 1–7°N latitude.

of tick typhus now includes Australia, Malaya, Thailand, Viet Nam, India, and probably Burma (Wilcocks 1944e).

Human tick typhus in Malaya was first detected in 1958 when investigators at the Institute for Medical Research (IMR) in Kuala Lumpur found Proteus OX-2 agglutinating and tick typhus complement-fixing antibodies in a patient suspected of having scrub typhus (IMR 1959). A diagnostic rise in titer to both the Proteus and complement-fixing antigens occurred in the absence of any reaction to murine-epidemic typhus or Q-fever antigens and no OX-19 or OX-k agglutinins were present. The following year additional human cases were found and the presence of complement-fixing antibodies in jungle animals, first reported by the IMR (1955), was established (IMR 1960).

This study has confirmed the serological evidence of tick typhus in Malayan wild life and demonstrated rickettsia in ticks as well as in wild and domiciliated mammals. Attempts have also been made to further elucidate the ecology of tick typhus in Malaya.

MATERIALS AND METHODS

Study Areas

The study was conducted in two major phases. The first was a general survey of the prevalence of tick typhus throughout Malaya in various forms of animal life with particular emphasis on wild vertebrates and their tick parasites. Collections were made from each of 11 ecologically distinct areas (ecosystems) in the states of Kedah, Penang, Perak, Selangor, Negri Sembilan, Johore and Malacca, all primarily in the western part of the Peninsula. These ecosystems and the animals collected from them are summarized in Table 4 and are fully described in Part 1 of this series.

The second phase of the study was an intensive survey of a limited area to determine the natural animal maintenance hosts and tick vectors of tick typhus rickettsiae in a natural forest cycle. A relatively undisturbed slope of hill dipterocarp forest was selected for study because it was accessible (the sample plots were approximately 1 km from the road), tick typhus had been demonstrated in the vicinity and it offered the opportunity to collaborate with Dr Robert Rudd, a University of California (Davis) zoologist studying the ecology of rodents in the same general area.

This area lies 34 km E of Kuala Lumpur at Ginting Sempah in the Ulu Gombak Forest Reserve (elevation approximately 540 m) in the central mountain chain extending most of the length of the Malay Peninsula. The habitat is tropical rain forest consisting of a climatic climax hill dipterocarp formation (the primary mixed dipterocrap forest of Richards (1957)). The dominant trees, belonging to the family Dipterocarpaceae, reach a height of over 45 m, are well separated and their discontinuous crowns form the upper quarter of four strata of vegetation. Below the dipterocarp crowns is a stratum of trees of many species and families, which forms a dense, nearly continuous canopy 15–30 m above the ground. A third stratum, also composed of many families, particularly the Annonaceae, grades into the one above contributing to the density of the canopy. On the forest floor are shrubs, palms, tree seedlings, ferns and herbs. The ground level vegetation is dense in spots but seldom as dense as the brush of many temperate zone forests.

The vegetation is lush and wet except on the steepest slopes. Bamboo thickets occur at irregular intervals on the hillsides, and wild bananas are scattered along the streams, whose water levels fluctuate greatly in response to short but frequent and heavy downpours, often occuring daily.

Two areas about 1 km apart were studied. In Area I, two grids of 160 traps each were set up and operated on alternate weeks from 7. X. 1963 until 1. V. 1964. The traps in each grid were placed in 8 parallel rows approximately 50 m apart and crossing the streams which flowed through each grid. Each row contained 20 traps placed approximately 10 m apart. Standard wire mesh live-traps were baited with banana and coconut or with sweet potato. Each animal trapped was placed in a cloth bag and the metal trap number attached. The day's catch was taken to the laboratory in Kuala Lumpur where ticks were removed from each specimen. Each animal was then bled by cardiac puncture under light ether anesthesia, and toe-clipped for identification. It was then placed in a small cage in a quiet place until the next morning when it was returned to the grid and released at the original trapping spot of the previous day.

Recaptured animals were handled in exactly the same manner as first captures except that they usually were not bled more often than once every 2 weeks.

Area II consisted of a nearby portion of the hill dipterocarp forest about 1 km from Area I. Almost every ground rodent in this area had been trapped and marked by Dr Rudd as part of an independent study. In April 1964, 89 of these marked animals were captured and processed as described above and released. One month later the same area was trapped again and 107 animals collected, including 34 animals trapped and released the preceding month.

Collection and Processing of Specimens

Phase I. An aborigine trapper assisted in the collection of small wild animals as described in Part 1.

Monkeys were obtained from a professional animal supplier. Animals were usually brought into the laboratory alive and processed immediately. Occasionally, however, when the distance from the laboratory was too great, they were processed on the spot and blood and tissues cooled on wet ice or frozen on dry ice for later shipment to the laboratory. Ticks were kept at ambient temperature during shipment.

Serum samples from domestic animals were obtained from the Veterinary Research Laboratory, Ipoh, and came primarily from herds in Johore. Serum samples from birds were obtained by toe clipping live-trapped birds and allowing the blood droplets to fall into small tubes containing 0.1 ml of sterile saline. Although crude, this bird serum was considered as a $\frac{1}{8}$ dilution and was collected by centrifugation in the usual manner. Human serum samples were obtained from various sources and were not systematically collected.

Live animals were brought to the laboratory usually on the day of capture, but occasionally not until the 2nd or 3rd day. Each animal was anesthetized and checked for ticks which were immediately identified, incubated at 37°C for 24 hr, and then stored at –60°C until inoculated into guinea pigs.

Each animal was bled aseptically while under ether anesthesia, then killed by overdose of anesthetic and autopsied. Duplicate portions of spleen, liver, kidney and lung were removed from each animal and stored at –60°C. No kidneys were obtained from most of the monkeys as they were used for other purposes. The blood was allowed to clot at room temperature, then placed in the refrigerator overnight. The serum was collected the following day and stored at –15°C.

Serology

All serum samples were tested by a micro complement-fixation technique utilizing a microtiter kit[9] (Sever 1962). Rickettsialpox and Rocky Mountain spotted fever (RMsf) antigens produced by Lederle were used to detect tick typhus antibody. The presence of antibody to Q-fever (Nine Mile strain) and psittacosis was determined with Lederle antigens. Antibody to group typhus was determined with a murine-epidemic typhus antigen produced by Markham Laboratories. Hemolysin was obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. Pooled normal guinea pig serum from at least 12 guinea pigs, each previously tested for the absence of rickettsial antibodies, was used for complement. It was stored in small vials at –60°C until needed. Two exact units of complement determined in the presence of antigen were used in the test. All the reagents were titrated before the performance of each test. Before testing, each serum sample was heated at 56°C for 30 min. Samples showing anti-complementary activity on the initial test were heated at 60°C for 30 min and the test repeated. Known positive and negative control sera were always included in each test. All serum samples fixing complement at a serum dilution of $\frac{1}{4}$ or higher in the presence of rickettsialpox antigen were retested and were considered positive only if essentially the same titer was obtained in at least two tests. Some rickettsialpox-positive serum samples also were tested with RMsf antigen. In addition all samples were tested for Q-fever and murine-epidemic typhus antibodies; and all tick typhus-positive samples were tested for psittacosis antibodies.

9. Cooke Engineering Co., Alexandria, Virginia.

Rickettsial Isolation

Stored tissue samples to be tested were thawed at room temperature and pooled by species and area of collection. The pools usually contained tissues from 4–5 animals, but occasionally there were as few as 1 and as many as 6 animals per pool. The tissues were ground with sterile sand in chilled mortars and made into 20% suspensions with sucrose-phosphate glutamate diluent (Bovarnick *et al* 1950), or with 1% bovine plasma albumin. The suspensions were allowed to stand in the refrigerator for approximately 30 min to allow the sand and large tissue fragments to settle. A portion of the supernatant was ampouled, quick-frozen and stored at –60°C for future reference. One ml of the remaining supernatant was inoculated intraperitoneally into each of two prebled adult 300–400 g guinea pigs; or occasionally into one guinea pig and two hamsters. A portion of each sample was also inoculated onto a blood agar plate and incubated at 37°C for detection of bacterial contaminants. The inoculated animals were observed daily for 28 days, then, if still alive, bled and discarded. The serum samples collected from each animal (pre- and post-inoculation) were tested for complement fixing rickettsial antibodies as described. Only those samples were considered positive in which the pre-inoculation titer was negative and the post-inoculation titer $\frac{1}{16}$ or higher. Tissue pools causing an antibody response in guinea pigs were reinoculated (from stored material) and observed as before, except that daily rectal temperatures of these animals were taken for 14 days after inoculation. An antibody rise again was accepted as presumptive evidence of the presence of rickettsiae in the tissue pool whether or not there was any febrile response.

Homogenates of tick-typhus positive pools were inoculated into the yolk sacs of $4\frac{1}{2}$ and 6 day old embryonated hens' eggs, and incubated at 35° and 37°C respectively. Up to 6 blind passages were made in

attempts to adapt the organisms to eggs.

Ticks pooled by species, host, and area were triturated with sterile sand in chilled mortars. Not over 60 ticks comprised a pool and the number usually was 10–20. The triturates were suspended in sucrose-phosphate-glutamate diluent containing 500 units each of penicillin and streptomycin per ml. Approximately 0.1 to 0.2 ml of diluent per tick was used, but occasionally up to 0.5 ml per tick had to be used when only a few ticks made up a pool. After allowing the sand and chitinous particles to settle in the refrigerator, a portion of each suspension was ampouled, quick-frozen and stored at −60°C, and the remainder inoculated intraperitoneally into each of two guinea pigs. The guinea pigs were observed as described for the tissues.

Table 4. Occurrence of tick typhus complement-fixing antibodies (to rickettsialpox antigen) in small wild mammals of Malaya.*

Species	Mountain Oak Forest	Upper Dipterocarp Forest	Hill Dipterocarp Forest	Lowland Dipterocarp Forest	Secondary Forest-Scrub	Freshwater Swamp Forest	Mangrove Swamp Forest	Islands	Estates	Agricultural land	Urban areas	Total	% Positive
Bandicota indica										0/1		0/1	0.0
Callosciurus caniceps			0/3**		0/1					0/3		0/7	0.0
C. hippurus			0/1									0/1	0.0
C. nigrovitatus			0/12	0/5	0/1							0/18	0.0
C. notatus	0/17		0/28	0/8	0/2	0/71	0/17	0/2	0/8			0/153	0.0
C. prevosti						0/3						0/3	0.0
C. tenuis				0/2		0/2						0/4	0.0
Chiropodomys gliroides			0/4									0/4	0.0
Lariscus insignis				0/1								0/1	0.0
Mus musculus											2/18	2/18	11.1
Rattus annandalei					0/2	0/17						0/19	0.0
R. bowersi			0/15	0/3	0/7							0/25	0.0
R. canus			0/8									0/8	0.0
R. cremoriventer	1/10		0/7	0/2	0/1							1/20	5.0
R. edwardsi	0/3	0/1										0/4	0.0
R. exulans					0/8	0/2	0/1	0/3	0/1	0/19	0/9	0/43	0.0
R. muelleri			0/44	0/4	0/12							0/60	0.0
R. norvegicus											0/38	0/38	0.0
R. rajah	3/14	4/8	1/12	2/15	0/2							10/51	20.0
R. rattus argentiventer										2/29	1/3	3/32	9.4
R. r. diardi							0/1		2/32	3/66	5/135	10/234	4.3
R. r. jalorensis					0/24		1/42	0/2	1/82	0/29		2/179	1.1
R. r. roa								1/63				1/63	1.6
R. sabanus			1/50	0/27	0/5							1/82	1.2
R. surifer	0/11	0/4	0/4					4/54				4/73	5.5
R. whiteheadi	1/25	0/3	0/6	0/18	1/18	0/9						2/79	2.5
Rhinosciurus laticaudatus			0/2			0/3						0/5	0.0
Echinosorex gymnurus			0/6	0/3	0/1	0/2	0/1					0/13	0.0
Hylomys suillus	0/1		0/2									0/3	0.0
Suncus murinus										0/1	15/92	15/93	16.1
Tupaia glis	5/25	1/6	1/10	1/19		0/1		2/27				10/88	11.4
T. minor			0/2	0/1								0/3	0.0
Total	10/106	5/22	3/216	3/108	1/84	0/110	1/62	7/151	3/123	5/148	23/295	61/1425	
% Positive	9.4	22.7	1.4	2.8	1.2	0.0	1.5	4.6	2.4	3.4	7.8	4.3	

* Only those samples showing no significant anticomplementary activity are included. A titer of 1/4 or greater is considered evidence for presence of tick typhus antibody in the serum sample.

** The number of serum samples fixing complement at a dilution of 1/4 or higher is shown in the numerator over the total number of samples tested in the denominator.

RESULTS

Phase I. General Survey

Tick typhus complement-fixing antibody was demonstrated in 4.3% (61/1425) of the small mammal serum samples that were not anticomplementary (Table 4). Approximately half the serum samples from *R. annandalei*, *R. bowersi* and *R. muelleri* were anticomplementary even after heating at 60°C for 30 min. The anticomplementary rate in *R. sabanus* and *R. surifer* was of the order of 10%, the remainder of the *Rattus* and other mammal sera tested was essentially free of anticomplementary activity (Table 5).

None of the tick typhus positive serum samples contained murine-epidemic typhus group or psittacosis antibody, nor did any contain Q-fever antibody with the exception of one *Tupaia glis*. About half the *T. glis* samples fixed complement in the presence of rickettsialpox antigen, but many of these reactions probably were nonspecific since only a portion also fixed complement in the presence of a Rocky Mountain spotted fever antigen (RMsf) (Table 6). Only those *T. glis* serum samples in this survey which fixed complement with both rickettsialpox and RMsf antigens are considered to possess specific tick typhus antibody.

The highest prevalence of tick typhus CF antibody in small wild mammals occurred in the upper-dipterocarp (22.7%) and mountain-oak forests (9.4%) of Gunong Jerai, a 1200 m mountain in Kedah, northern Malaya. All these samples were collected at elevations between 750 and 1170 m.

In the hill-dipterocarp and lowland-dipterocarp forests of the main range, only 1.4% and 2.8% respectively of the animals tested possessed tick typhus antibody. In contrast 7.8% of the animals collected from urban areas were positive.

The proportion of tick typhus positive animals in the four climatic climax ecosystems combined (mountain-oak, upper dipterocarp, hill dipterocarp, lowland dipterocarp) is 6.1%, approximately the same as the proportion of positive animals (5.2%) in the heavily man-disturbed ecosystems (estates, agricultural, urban) (Table 7). In the edaphic climax ecosystems (freshwater swamp and mangrove swamp) and the secondary forest-scrub-lalang ecosystems, the proportion of tick typhus positive small mammals is only 1.5%. In the insular ecosystem, which is in reality a mixture of climatic climax forest formations and man-disturbed habitats, the proportion of tick typhus positive mammals is 4.6% (Table 4). In Table 7 the animals from the insular habitat are included in their appropriate ecosystems (e.g., climatic climax forests and agricultural).

Complement-fixing antibody ranging in titer from $\frac{1}{4}$ to $\frac{1}{8}$ was demonstrated in 12 of 166 *Macaca irus* collected from lowland dipterocarp forest. Three of 74 *Presbytis cristatus*, mostly from mangrove swamp and lowland dipterocarp forests, had titers of $\frac{1}{4}$ to $\frac{1}{16}$. One of the two *M. nemestrina* collected in northern Malaya had a titer greater than $\frac{1}{16}$. None of the other

Table 5. Anticomplementary (AC) activity in the serum of *Rattus* species.

Species	No. Tested	No. AC*	% AC
Rattus annandalei	47	19	40.4
R. bowersi	58	33	56.9
R. canus	8	0	0.0
R. cremoriventer	20	0	0.0
R. edwardsi	5	1	20.0
R. exulans	48	0	0.0
R. muelleri	105	45	42.8
R. norvegicus	40	2	5.0
R. rajah	52	1	0.5
R. rattus argentiventer	32	0	0.0
R. r. jalorensis	189	10	5.3
*R. rattus***	297	0	0.0
R. sabanus	91	9	10.0
R. surifer	81	9	11.1
R. whiteheadi	79	0	0.0
Total	1152	129	11.2

* Specimens were considered irreversibly anticomplementary if the sample fixed complement at a dilution of 1/8 or greater after heating at 60°C for 30 min.

** *Rattus r. diardi* and *R. r. roa*.

Table 6. The fixation of complement by *Tupaia glis* serum in the presence of murine-epidemic typhus group, rickettsialpox, Rocky Mountain spotted fever (RMsf) and Q-fever antigens.*

Nature of Reaction	No. of samples reacting	% reactors
Reacted to none of the antigens	43	49.0
Reacted to murine-epidemic typhus antigen	0	
Reacted only to Q-fever antigen	2	2.3
Reacted only to rickettsialpox antigen	29	33.0
Reacted only to rickettsialpox and Q-fever	4	4.6
Reacted only to rickettsialpox and RMsf**	9	10.0
Reacted to rickettsialpox, RMsf and Q-fever	1	1.1
Total number of serum samples tested	88	100.0

* Serum samples fixing complement at a serum dilution of 1/4 or higher are considered to be positive reactors.

** None of the samples tested reacted to RMsf antigen only. All samples fixing complement with RMsf antigen also fixed complement with rickettsialpox antigen at the same or higher titer.

Table 7. Summary of tick typhus serological results in terms of major ecosystem complexes.*

Species	Climatic Climax Forests	Scrub-Edaphic Climax Forests	Urban-Semi Urban	Total	%
Mus musculus			2/18**	2/18	11.1
Rattus annandalei		0/19		0/19	0.0
R. bowersi	0/18	0/7		0/25	0.0
R. canus	0/8			0/8	0.0
R. cremoriventer	1/19	0/1		1/20	5.0
R. edwardsi	0/4			0/4	0.0
R. exulans		0/11	0/32	0/43	0.0
R. muelleri‡	0/48	0/12		0/60	0.0
R. norvegicus			0/38	0/38	0.0
R. rajah	10/49	0/2		10/51	20.0
R. rattus argentiventer‡			3/32	3/32	9.4
R. r. diardi‡		0/1	10/233	10/234	4.3
R. r. jalorensis		1/66	1/113	2/179	1.1
R. r. roa			1/63	1/63	1.6
R. sabanus	1/77	0/5		1/82	1.2
R. surifer	4/73			4/73	5.5
R. whiteheadi‡	1/52	1/27		2/79	2.6
Suncus murinus			15/93	15/93	16.2
Tupaia glis	8/60	0/1	2/27	10/88	11.4
T. minor	0/2	0/1		0/3	0.0
Total	25/410	2/153	34/649	61/1212	
%	6.1	1.5	5.3	5.0	

* Climatic climax forests consist of mountain-oak, upper dipterocarp, hill dipterocarp and lowland dipterocarp forests. Scrub-edaphic climax forests consist of secondary forest-scrub, freshwater swamp and mangrove swamp forests. Urban-semiurban consists of estates, agricultural land and urban areas.
Animals in the island ecosystem have been assigned to the appropriate categories above depending on whether they were trapped in climax forest or urban areas on the islands.

** The numerator shows the number of samples fixing complement at a serum dilution of 1/4 or higher in the presence of rickettsialpox antigen; the denominater shows the total number tested.

‡ Denotes species from which presumptive isolations of tick typhus rickettsiae were made.

48 mammals tested had any demonstrable tick typhus antibody (Table 8).

None of the 53 goat nor 322 pig serum samples contained tick typhus antibody. Four of the 435 cattle serum samples were positive with titers ranging from $\frac{1}{8}$ to $\frac{1}{16}$.

Eight of the 216 human samples were positive at a titer of $\frac{1}{4}$.

Relatively high tick typhus antibody titers of $\frac{1}{16}$ to $\frac{1}{64}$ were detected in four ground or brush dwelling species of bulbuls (Table 8). Two of the positive birds were collected in estates (large olive bulbul and yellow vented bulbul) and two in scrub (crested brown bulbul and white-throated bulbul). In the remaining 40 bird serum samples the antibody titer was less than $\frac{1}{8}$, the lowest dilution tested.

In general the antibody level in Malayan animals was relatively low although many had titers of $\frac{1}{16}$ or over (Table 9). Antibody levels in guinea pigs inoculated with positive tissue samples never rose higher than $\frac{1}{256}$ and were generally $\frac{1}{16}$ - $\frac{1}{64}$.

Presumptive isolations of tick typhus rickettsiae were made from 4 of the 433 pools of tissues from 1,849 mammals. The positive pools contained tissues of animals collected from hill dipterocarp, lowland dipterocarp and agricultural ecosystems (Table 10). In all cases the only evidence of infection was a rise in antibody titer from negative to $\frac{1}{16}$ or higher in guinea pigs 4 weeks after inoculation with tissue homogenate. There was no febrile response nor any scrotal reaction. Attempts to establish these presumptive isolates in eggs and to pass them in guinea pigs were not successful.

In addition to the above four isolations, there was some evidence that tick typhus rickettsiae were present in two pools of tissues from *T. glis* (1 from mountain-oak; 1 from insular ecosystems), one pool of *R. muelleri* tissue (hill-dipterocarp) and one pool of tissues from *C. notatus* (mangrove swamp). Initial inoculation of these pools caused weak antibody responses in one or both guinea pigs, but caused no antibody response (or at most a low response in one guinea pig) upon reinoculation.

No tick typhus rickettsiae were recovered from any

Table 8. Tick typhus complement-fixing antibody in monkeys, other large wild mammals and birds*** of Malaya.

Species	No. Collected	No. AC*	No. Positive**	% Positive
Macaca irus (Cuvier), Long-tailed (crab-eating) macaque	166	4	12	7.3
M. nemestrina (L.), Pig-tailed macaque	2		1	50.0
Presbytis cristatus (Raffles), Silvered leaf monkey	74	2	3	4.0
P. melalophos (Raffles), Banded leaf monkey	4			
Manis javanica Desmarest, Pangolin (Scaly anteater)	1			
Cynocephalus variegatus (Audebert), Flying lemur	1			
Pteropus hypomelanus Temmink, Lesser flying fox	5			
Tragulus javanicus (Osbeck), Smaller mouse deer	36			
Sus scrofa L., Common wild pig	1			
BIRDS				
Malacopteron cinereum Eyton, Lesser red-headed babbler	1			
Stachyris maculata (Temminck), Red-rumped tree babbler	1			
S. nigricollis (Temminck), Black-necked tree babbler	2			
S. erythroptera (Blyth), Red-winged tree babbler	1			
Trichastoma malaccensis (Hartlaub), Short-tailed babbler	1			
Criniger phaeocephalus (Hartlaub), White-throated bulbul	1		1	100.0
Hypsipetes criniger (Blyth), Hairy-backed bulbul	1			
Pycnonotus goiavier (Scopoli), Yellow-vented bulbul	6		1	16.7
P. plumosus (Blyth), Large olive bulbul	14		1	7.1
P. eutilotus (Jardine & Selby), Crested brown bulbul	1		1	100.0
Picus vittatus Vieillot, Bamboo green woodpecker	1			
Ceyx erithacus (L.), Black-backed kingfisher	2			
Anthreptes malacensis (Scopoli), Brown-throated sunbird	3			
Pitta sordida (Muller), Hooded-pitta	1			
Chloropsis cyanopogon (Temmink), Lesser green leaf bird	2			
Otus scops (L.), Scops owl	1			
Luscinia cyane (Pallas), Siberian blue robin	1			
Copsychus saularis (L.), Straits (magpie) robin	2			
Amaurornis phoenicurus (Pennant), White-breasted waterhen	2			
Total	334	6	20	

* Number anticomplementary.

** Number of serum samples fixing complement at serum dilutions of 1/4 or higher in the presence of rickettsialpox antigen.

*** The bird names are based on Medway & Wells (1964) and on Smythies (1953, 1960).

of the 33 pools of 1,175 ticks collected and processed during this survey.

Phase II. Intensive Study

In Study Area I in the hill-dipterocarp forest at Ulu Gombak, 70% of the animals captured were *R. sabanus*. The only other species present in relatively large numbers were *R. muelleri*, reflecting the presence of streams, and *T. glis*. These three species comprised 88% of the ground-dwelling small mammal population. Five of seven *R. edwardsi* and 3 of 19 *T. glis* and approximately $\frac{1}{3}$ of the *R. sabanus* and *R. muelleri* were retrapped at least once during the 7-month study (Table 11).

The only animals with tick typhus antibody were 2 *R. edwardsi* and 5 *T. glis* (Table 12). A rise in antibody titer from 0 to $\frac{1}{8}$ three months later was demonstrated in one of the *R. edwardsi*. The other one had a relatively high initial CF titer of $\frac{1}{64}$, indicating a recent infection.

Three *T. glis* captured only once had titers of $\frac{1}{4}$, $\frac{1}{4}$ and $\frac{1}{64}$ respectively. Tick typhus and Q-fever antibody titers in four others recaptured one or more times are shown in Table 13.

Dermacentor auratus was the most abundant tick, followed in order by *Ixodes granulatus* and *Haemaphysalis* spp.[10] Only three *Amblyomma* spp. were collected (Table 14). The animals with the most ticks were *R. muelleri* (which also was heavily infested with mesostigmatic mites), *R. sabanus*, and *Rhinosciurus laticaudatus*. The overall tick index of those animals from which ticks were collected was 1.6.

Presumptive isolations of tick typhus rickettsiae

10. Probably *H. papuana* Thorell and *H. semernis* Neumann according to Nadchatram (1965).

were made from 6 of 29 tick pools. Five of the positive pools contained *I. granulatus* collected from *R. sabanus*, *R. edwardsi*, *R. muelleri*, *R. annandalei* and *R. rajah* (Table 15). None of the guinea pigs into which the tick pools were inoculated showed a febrile response nor a scrotal reaction. The only evidence of infection with tick typhus rickettsiae was the conversion of antibody titer from 0 to $\frac{1}{16}$ or higher 4 weeks after inoculation.

Table 9. Levels of tick typhus complement-fixing antibody in mammals of Malaya.

Species	No. of specimens with antibody titer* of		
	1/4	1/8	1/16
Mus musculus	2		
R. cremoriventer	1		
R. rajah	3	3	4
R. rattus argentiventer	2	1	
R. r. jalorensis	1		1
*R. rattus***	7	2	2
R. sabanus			1
R. surifer	1	1	2
R. whiteheadi	1	1	
Suncus murinus	10	3	2
*Tupaia glis****	3	1	6
Presbytis cristatus	1	1	1
Macaca irus	5	1	4
M. nemestrina			1
Total	37	14	24

* Highest serum dilution fixing complement in the presence of rickettsialpox antigen.

** *Rattus r. diardi* and *R. r. roa*.

*** In some cases the titers obtained with RMsf antigen in the same samples were slightly lower.

In Study Area II (trapped for 1 week in April and 1 week in May, 1964), *R. sabanus* comprised only 44% of the catch. However, it was over twice as abundant as any other species in this area where 16 small mammal species were collected in 2 weeks trapping compared to 13 species in 7 months trapping in Study Area I. Three species (*R. rajah*, *R. sabanus* and *R. surifer*) comprised 74% of the catch.

Tick typhus antibody was demonstrated in one *Chiropodomys gliroides*, one *R. edwardsi* and one *R. sabanus* (Table 16).

As in Study Area I, *Dermacentor auratus* was the most abundant tick collected, followed in order by *I. granulatus* and *Haemaphysalis* spp. No *Amblyomma* were collected. The mammals with the most ticks per animal were *R. edwardsi*, *R. annandalei* and *R. sabanus*. The overall tick index among those animals with ticks was 0.9 (Table 17).

No presumptive isolations of tick typhus rickettsiae were made from ticks collected from this study plot.

Table 10. Mammals from which presumptive isolation of tick typhus rickettsiae have been made in Malaya.*

Host species	Locality	Ecosystem
Rattus muelleri	Ulu Gombak	Hill-dipterocarp forest
R. whiteheadi	Kuang	Lowland-dipterocarp forest
R. r. diardi	Malacca	Agricultural land
R. r. argentiventer	Malacca	Agricultural land

* In each case the presumptive isolations were made from 1 pool containing tissues from 4–5 animals of the species listed.

Table 11. Animals collected from Phase II, Study Area I at Ulu Gombak.

Species	Total No. of individuals captured	No. of animals captured				No. dying during study
		1 time only	2 times only	3 times only	4 or more times	
Rattus annandalei	5	3			2	
R. bowersi	6	6				
R. canus	1	1				
R. edwardsi	7	2	2	1	2	
R. muelleri	24	16	6	2		
R. rajah	2	2				
R. sabanus	180	118	39	10	13	9
R. surifer	3	3				1
R. whiteheadi	5	5				
Rhinosciurus laticaudatus	1	1				
Echinosorex gymnurus	2	2				2
Hylomys suillus	5	5				3
Tupaia glis	19	16	2		1	3
Total	260	180	49	13	18	18

Table 12. Tick typhus complement-fixing antibody titers in serum samples taken from animals each time captured from Intensive Study Area I. Also shown is the number of samples that were anticomplementary at each bleeding.

Species	No. of serum samples tested	No. AC each bleeding (Total No. tested) 1st	2nd	3rd	4th	No. tick typhus positive* (Total No. tested) 1st	2nd	3rd	4th
Rattus annandalei	15	0/5	0/2	1/2	1/6	0/5	0/2	0/1	0/5
R. edwardsi	17	0/7	0/5	0/2	0/3	1**/7	1***/5	0/2	0/3
R. muelleri	36	6/24	2/9	2/3	—	0/18	0/7	0/1	—
R. sabanus	283	24/180	26/62	14/23	9/18	0/156	0/36	0/9	0/9
Tupaia glis	27	0/19	0/4	0/1	0/3	5/19	4/4	1/1	3/3
Total	378	30/235	28/82	17/31	10/30	6/205	5/54	1/14	3/20
%		12.8	34.2	54.8	33.3	2.9	9.3	7.1	15.0

* Anticomplementary serum samples not included. Samples fixing complement at a serum dilution of 1/4 or higher in the presence of rickettsialpox antigen are considered positive for tick typhus antibody.

** First bleeding titer was 1/64; the animal was not recaptured.

*** Titer was negative at first bleeding and 1/8 on second bleeding three months later.

Table 13. Tick typhus and Q-fever complement-fixing antibody titers* in *Tupaia glis* captured and bled more than once in Intensive Study Area I.

Animal No.	Tick typhus CF titer Sample 1	Sample 2	Sample 3	Sample 4	Q-fever CF titer Sample 1	Sample 2	Sample 3	Sample 4
3	4	16(2)**			neg	4		
12	neg	16(13)	16(2)	32‡(30)	neg	neg	neg	neg
15	neg	16(35)			neg	neg		
16	16	32(3)			4	8		

* Titers are shown as the reciprocal of the highest dilution showing 50% lysis of sheep red cells in the presence of complement and rickettsialpox antigen.

** In parenthesis is the number of days since the previous sample was collected.

‡ Also $\frac{1}{32}$ on 5th and 6th bleedings 2 and 23 days respectively after the 4th bleeding; Q-fever titer remained negative.

Table 14. Ticks collected from animals trapped in Intensive Study Area I.

Host Species	No. of Animals Collected	*Haemaphysalis* spp. L‡	N	*Ixodes granulatus* N	A	*Dermacentor auratus* L	N	*Amblyomma* sp. L	N	Total	Tick Index
Rattus annandalei	15*				14		5			19	1.3
R. bowersi	6		4	1			3			8	1.3
R. canus	1									0	0.0
R. edwardsi	17♯		1		4		12			17	1.0
R. muelleri	34**		4		72	1	25			102	3.0
R. rajah	2		1			4	1			6	3.0
R. sabanus	229***	88	38		54	107	81			368	1.6
R. surifer	2									0	0.0
R. whiteheadi	5									0	0.0
Rhinosciurus laticaudatus	1		17		2					19	19.0
Echinosorex gymnurus	2				1					1	0.5
Hylomys suillus	5									0	0.0
Tupaia glis	19		1	1			3		3	8	0.4
Total	338	88	66	2	147	112	130		3	548	1.6

‡ Larvae (L), Nymphs (N), Adults (A).

* Includes 10 recaptures.

** Includes 10 recaptures. 49 of the ticks were taken from *R. muelleri* captured the first time and 53 on animals recaptured two or more weeks later.

*** Includes 49 recaptures.

♯ Includes 10 recaptures.

DISCUSSION

The serological results demonstrate the presence of tick typhus in the wildlife of Malaya and confirm the widespread occurrence of tick typhus in wild animals suggested by earlier workers (IMR 1960).

Analysis of the data, however, shows that the distribution of tick typhus complement-fixing antibody is not uniform over the entire range of species or habitats. It was not detected in the nearly 300 squirrels tested (mostly *Callosciurus* and *Rhinosciurus*). Earlier workers in Malaya (IMR 1959, 1960) reported the same negative results. The squirrels are parasitized mainly by *Dermacentor auratus* (see Part 1; Audy *et al* 1960), which has not been shown to harbor tick typhus rickettsiae. *Ixodes granulatus*, a known vector, is rarely found on them. In the absence of data incriminating squirrels in tick typhus ecology, they are not assumed to be an important link in the cycle of Malayan tick typhus. The animals involved are the forest rats and tree shrews and the urban rats and house shrews.

Table 7 shows that tick typhus activity is most prevalent in two ecosystem complexes: the climatic climax forests remote from human interference and urban situations where man is the dominant environmental factor.

In the climatic climax forests of Malaya, *Rattus rajah*, *R. cremoriventer*, *R. surifer* and *Tupaia glis*, and their *Ixodes granulatus* ticks are of prime importance

Table 15. Presumptive isolation of tick-typhus rickettsiae from *Ixodes granulatus* and *Haemaphysalis* spp. collected from rodents trapped in hill-dipterocarp forest at Study Area I.

Tick Pool No.	Composition of Pool	Rodent Host
T-40	27 adult *Ix. granulatus*	15 *R. sabanus*
T-49	26 adult *Ix. granulatus*	14 *R. sabanus*
	4 adult *Ix. granulatus*	2 *R. edwardsi*
T-65	4 Nymphal *Haemaphysalis* spp.	1 *R. bowersi*
T-79	1 Larval, 2 Nymphal and 28 adult *Ix. granulatus*	7 *R. muelleri*
		2 *R. annandalei*
T-80	2 adult *Ix. granulatus*	2 *R. rajah*
T-83	15 adult *Ix. granulatus*	4 *R. annandalei*

Table 16. Tick Typhus Complement-Fixing antibody in animals captured once only in the Intensive Study Area II.*

Species	No. Serum Samples	No. Samples AC	No. with Tick Typhus Antibody**
Callosciurus caniceps	3		
C. nigrovitatus	1		
C. notatus	1		
C. tenuis	5		
Chiropodomys gliroides	3		1
Rattus annandalei	2	1	
R. cremoriventer	5		
R. edwardsi	4	2	1
R. r. jalorensis	1		
R. muelleri	8	3	
R. rajah	20	3	
R. sabanus	70	18	1
R. surifer	28	4	
R. whiteheadi	7		
Hylomys suillus	1		
Tupaia minor	1		
Total	160	31	3

* In addition to the animals presented, 34 were recaptured and bled 1 month after initial capture: 20 *R. sabanus*, 5 *R. surifer*, 5 *R. rajah*, and 1 each *R. annandalei*, *R. edwardsi*, *R. muelleri* and *R. cremoriventer*. No antibodies were detected in any of these animals on the second bleeding.

** Samples fixing complement at a serum dilution of 1/4 or higher in the presence of rickettsialpox antigen are considered to possess tick typhus antibody.

Table 17. Ticks collected from animals trapped in April and May, 1964 from Intensive Study Area II.

Host Species	No. of Animals Collected*	*Haemaphysalis*		*Ixodes granulatus*			*Dermacentor auratus*		Total Ticks	Tick Index
		L	N	L	N	A	L	N		
Rattus annandalei	2					5			5	2.5
R. edwardsi	4		2			1		10	13	3.3
R. muelleri	8			1	2	5			8	1.0
R. rajah	20				1	1			2	0.1
R. sabanus	70		14	1	1	27	41	4	88	1.3
R. surifer	28		1			5	1	1	8	0.3
R. cremoriventer	5							2	2	0.8
R. r. jalorensis	1							1	1	1.0
Total	138	0	17	2	4	44	42	18	127	0.9

* Includes the following number of recaptures: *R. annandalei* 1, *R. edwardsi* 1, *R. muelleri* 1, *R. rajah* 5, *R. sabanus* 20, *R. surifer* 5, *R. cremoriventer* 1.

in maintaining the tick typhus cycle. In this respect Malayan tick typhus resembles Rocky Mountain spotted fever (Burgdorfer *et al* 1962) and the tick typhus rickettsioses of Kenya (Heisch *et al* 1962), S. Africa (Gear 1954; Neitz 1956), Siberia (Zdrodovskii & Golinevich 1960), and probably those of North Queensland (Philip 1963), in all of which wild mammals and their ticks play a prominant role.

The urban cycle of Malayan tick typhus, on the other hand, resembles boutonneuse fever and Indian tick typhus in both of which forest animals are not involved. Few ticks are found on urban rats and house shrews, but Malayan dogs are heavily infested with *Rhipicephalus sanguineus*, an important vector of boutonneuse fever in the Mediterranean area of southern and southeastern Europe and northwest Africa and of tick typhus in India (Hoogstraal 1961). House shrews and house rats could conceivably pick up the rickettsiae from infected dog ticks, perhaps by eating them. Unfortunately this must remain only a possibility until more complete investigation has been made.

If tick typhus exists in a distinct urban cycle as the data suggest, the rickettsiae could be transmitted by mesostigmatic mites as is *Rickettsia akari* in the United States. Isolations of *R. sibericus* also have been reported from gamasid mites in the Soviet Union (Shapiro 1958), but this need not imply that they are capable of transmitting the rickettsiae. Several pools of mesostigmatic mites from *Suncus murinus* and *Rattus r. diardi* trapped in Kuala Lumpur were tested, but no rickettsial isolations were made.

Without specific evidence, the urban tick typhus cycle in Malaya cannot be established as an independent, self-sustaining cycle. However, to postulate such an independent cycle is not necessary to explain the high prevalence of apparent infections in urban and other domiciliated mammals. Ample opportunity exists for indirect transmission of rickettsiae from forest to urban animals. Some forest species (particularly *Rattus whiteheadi*, *R. bowersi* and *R. muelleri*) extend into man-disturbed secondary forest and scrub habitats where man-associated species (*Rattus r. jalorensis*, *R. exulans* and *R. r. argentiventer*) also are regularly found. These animals of the secondary forest-scrub, estate and agricultural ecosystems in turn often come into contact with such truly urban species as *R. r. diardi* and *S. murinus* (Harrison 1957; see Part 1).

There is also evidence that birds may be an important link between forest and scrub species of animal carriers of rickettsiae. A number of scrub and secondary forest inhabiting birds tested possessed relatively high antibody titers, especially the bulbuls. Ground birds usually carry *Haemaphysalis* ticks but may be host to others as well (Audy *et al* 1960). Unfortunately there is no information on the tick fauna of bulbuls.

Evidently there is a continuous path over which parasites may be transmitted from forest to urban ecosystems, and the postulation of an independent urban cycle of tick typhus may be unnecessary.

A situation apparently similar to that in Malaya exists in South Africa where a high prevalence of tick typhus antibody in urban *Rattus rattus* exists, particularly in suburban Johannesburg, in addition to the known natural focus among veld rodents. Isolation of a strain of *Rickettsia conori* from an urban rat was reported by Gear (1954). In his opinion, however, these domiciliated rats, like man, probably are infected by ticks accidentally picked up on the veld where natural foci occur among the wild animals.

The very low prevalence of tick typhus antibody in cattle and its absence in goats and pigs indicates that these animals probably play little or no role in Malayan tick typhus. Domestic farm animals generally have not been implicated in tick typhus cycles, but reports from Mexico (Silva-Goytia & Elizondo 1953) indicate that cows, donkeys and goats may acquire inapparent infections and harbor infected ticks. Heisch *et al* (1957) also isolated tick typhus rickettsiae from a batch of *Amblyomma variegatum* taken from cattle in Nairobi. Domestic animals in any area of tick typhus activity may serve as hosts to adult vector ticks and thus become involved secondarily in the enzootic cycle, but there is little evidence that they ever become so intimately involved as they do with Q-fever.

A curious aspect of tick typhus in Malaya is the extremely low virulence of the organism. On initial isolation in guinea pigs, no febrile period or scrotal reaction could be demonstrated. Attempts to serially pass the organism in guinea pigs and hamsters were unsuccessful. On one occasion a strain apparently was carried through three passages in guinea pigs, then lost. Attempts to pass it in embryonated hens' eggs fared no better. No strains were adapted to eggs through six passages. However, difficulties in obtaining antibiotic-free embryonated eggs forced premature abandonment of this phase of the study.

Low virulent strains of *R. rickettsii* in the U.S. (Price 1953), and of Indian tick typhus (Browing & Kalra 1948) have been isolated from nature; and Pope (1955) reported the low virulence of strains of *R. australis* in South Queensland. In the latter case initial and subsequent blind passage in guinea pigs produced no overt signs of disease, but low titer ($\frac{1}{8}$ to $\frac{1}{16}$) CF antibodies were formed. However, Pope (*l.c.*) found that if the organism was first isolated in mice and then inoculated into guinea pigs, a typical febrile response and scrotal reaction was produced, suggesting that the mouse may be a more suitable

animal for isolation of low virulent tick typhus strains.

The low virulence of the Malayan tick typhus rickettsiae may explain the extremely low prevalence of disease in humans. It also suggests, along with the other data presented, that the rickettsiae and its tick and mammal hosts are well adapted to each other and have had a long evolutionary history.

SUMMARY AND CONCLUSIONS

In a survey of the prevalence of tick typhus in Malaya, 1715 small mammals, monkeys and larger animals, 44 birds and several hundred cattle, pigs and goats were tested serologically for presence of complement-fixing antibodies against tick typhus. Tick typhus CF antibody was demonstrated in 4.3% of the small mammal serum samples that were not anticomplementary. The greatest proportion of positive samples came from *R. rajah*, *S. murinus*, *M. musculus* and *Tupaia glis*.

Tick typhus CF antibody was demonstrated in 16 of the 246 monkey serum samples tested (*Macaca irus*, *Presbytis cristatus* and *M. nemistrina*), but none of 44 other mammals of various species had any demonstrable antibody. No tick typhus antibody was demonstrable in goats or pigs, but 4 of 435 cattle were found to have titers ranging from $\frac{1}{8}$ to $\frac{1}{16}$.

Eight of 216 human serum samples tested were positive at a titer of $\frac{1}{4}$.

Relatively high tick typhus antibody titers of $\frac{1}{16}$ to $\frac{1}{64}$ were demonstrated in four ground or brush dwelling species of birds collected from estates and scrub habitats.

The highest prevalence of tick typhus antibody occurred in wildlife samples collected from upper-dipterocarp (22.7%) and mountain oak (9.4%) forests at elevations above 750 m. In the lowland dipterocarp forests on the Island of Pulau Aur and in the main range, 2.8% of the animals collected had tick typhus CF antibody. In strictly urban areas, nearly 8% of the animals tested had antibodies to tick typhus.

Presumptive isolations of tick typhus rickettsiae were made from 4 of 433 pools of tissues from 1849 wild mammals. The positive pools contained tissues of animals collected in hill dipterocarp (*R. muelleri*), lowland dipterocarp (*R. whiteheadi*) and agricultural (*R. r. argentiventer* and *R. r. diardi*) ecosystems.

Tick typhus rickettsiae were isolated from ticks (*Ixodes granulatus* and *Haemaphysalis* spp.) collected from an area of hill dipterocarp forest which was investigated intensively for seven months. Six of the 29 pools of ticks tested were positive for tick typhus rickettsiae—5 pools of *I. granulatus* and 1 pool of *Haemaphysalis* spp.

In Malaya there are apparently urban and forest cycles of tick typhus activity. However, the urban cycle is probably secondary to the forest cycle and the animals living in man-dominated habitats receive their infections indirectly from forest rodents. Domiciliated, urban rodents may acquire tick typhus infection from infected ticks initially picked up by secondary forest-scrub rodents, which in turn acquired them from contact with forest animals. The serological evidence of tick typhus infection in scrub inhabiting rodents is scant, but shows that secondary forest and scrub birds may harbor tick typhus rickettsiae in these habitats.

In the forest, the most likely cycle involves the giant and spiny-furred forest rats (*R. rajah*, *R. surifer*, *R. sabanus*, *R. muelleri*, *R. edwardsi*, etc.) and *Ixodes granulatus* and possibly *Haemaphysalis* spp. ticks. The tree shrews may be included in the cycle, but solely on serological evidence. There is no direct evidence that *Dermacentor* ticks are involved. On the basis of abundance, host range and rickettsial isolations, *I. granulatus* appears to be the most important tick in the primary forest cycle of tick typhus.

PART 3. Q-FEVER

The presence of Q-fever in Malaya was not recognized until 1951, when a serological survey revealed complement fixing antibody against *Coxiella burneti* in man and domestic animals (IMR 1951). Subsequently, sporadic human cases were diagnosed (IMR 1957, 1959, 1960), and Q-fever antibodies were also found in the sera of wild rodents (IMR 1955), In the first major study of Q-fever in Malaya, conducted by personnel of the U.S. Army Medical Research Unit at the Institute for Medical Research in Kuala Lumpur, *C. burneti* was isolated from pools of *Haemaphysalis* and *Ixodes* ticks collected from forest rodents (IMR 1959, 1960).

Coincident with the report of Q-fever in man in Malaya, Clark *et al* (1951) reported an outbreak of the disease among goats on a cargo ship bound for the Far East. Although Q-fever is known to have spread by transport of domestic animals, it is not justifiable to assume that it initially arrived in Malaya in that manner. *Coxiella burneti* probably has long existed there, as it has in Australia, the United States, and many other countries (Derrick 1944). Our limited

knowledge of Q-fever in the tropics, as Philip (1963) pointed out, is probably due to lack of survey rather than a lack of rickettsiae.

Not until after World War II was attention turned to the possibility of Q-fever occurring in the Asian region. Coincident with its discovery in Malaya, Chang *et al* (1951) reported from China a case of primary atypical pneumonia in which there was a diagnostic rise in Q-fever antibody titer. In Ceylon, a serological survey demonstrated the prevalence of Q-fever antibodies among abattoir workers in Colombo and in buffaloes, goats and sheep throughout the island (Schmid *et al* 1952). Serological surveys and the occasional occurrence of cases have demonstrated the endemicity of Q-fever in India in man and domestic animals (Kalra & Taneja 1954; Soman 1954; Veeraraghavan & Sukumaran 1954; Andersen & Kalra 1954). Surveys in Japan about the same time revealed a similar situation (Takano *et al* 1954). In 1954 there was an outbreak of Q-fever among the French naval crews in Saigon, Vietnam, but the origin of the epidemic remains unknown (Quintin *et al* 1955). The prevalence of Q-fever in Vietnam itself is also unknown as it is in Indonesia, although serologically positive bovines have been reported there (Kaplan & Bertagna 1955). There are no published reports of Q-fever in Burma, Thailand, Laos, Cambodia, or the Philippines, where it is probably present but may not reach epidemic proportions or cause severe human illness.

It is now well established that Q-fever is endemic in SE Asia. Little is known of its ecology in this area or in tropical regions in general, but wild animals probably maintain the basic cycles as they do in other parts of the world.

The evidence for primary cycles of *C. burneti* in wild animals has been accumulating rapidly since Derrick & Smith (1939) discovered one such cycle in bandicoots (*Isodon torosus*) and ticks (*Haemaphysalis humerosa*) on Moreton Island off the coast of Australia. Partly on the basis of this work, Derrick (1944) proposed that *C. burneti* was probably indigenous to the Queensland bush and antedated the arrival of man and his domestic animals, its primary cycle occurring in several species of ticks and their bandicoots, and other small bush animal hosts. Domestic animals and man became secondarily involved. Subsequently, this hypothesis was supported by Dwyer *et al* (1960) who found a similar cycle in another species of bandicoot, *I. macrourus*, and by Pope *et al* (1960) who described an apparent Q-fever cycle in kangaroos and the tick, *Amblyomma triguttatum*.

Further evidence of *C. burneti* cycles in wild animals in Kenya was reported by Heisch (1960). There are also reports of Q-fever in forest and desert rodents in Morocco (Blanc & Bruneau 1956; Blanc *et al* 1947) and in the western United States (Burgdorfer *et al* 1963; Stoenner *et al* 1959; Sidwell *et al* 1964). Dormice and mountain rabbits in Spain (Perez-Gallardo *et al* 1952) and many wild animals in Czechoslovakia (Raska *et al* 1956) and Russia (Zdrodovskii & Golinevich 1960) have been found infected with *C. burneti*. In addition, Syrucek (1959) in Czechoslovakia observed that Q-fever did not persist when introduced by imported domestic animals into areas free of ticks, demonstrating the importance of the latter in the maintenance of *C. burneti*.

Domestic ruminants are now a major reservoir of the rickettsiae in many parts of the world where there are ticks, and the infection probably spills over from them to wild animals (Marmion 1959). This, however, does not weaken the hypothesis that wild animals and their ticks are the basic and original reservoir and the main system of maintenance.

The purpose of this study was to determine the prevalence, distribution, and natural cycles of *C. burneti* in Malaya.

MATERIALS AND METHODS

This investigations was carried out in two phases. Phase I consisted of a general survey of Q-fever in domestic, domicilated[11] and wild animals in Malaya. The localities studied and the animals, their habitats, the methods of collecting and the processing of tissue and serum samples are described in Parts 1 and 2, and will not be discussed here except where specific techniques differed.

All serum samples were tested for complement-fixing (CF) antibodies to rickettsialpox and murine-epidemic typhus (Parts 2 & 3) in addition to Q-fever; and all Q-fever positive samples were tested for psittacosis antibodies. The murine-epidemic typhus and psittacosis antigens were used primarily as controls for nonspecific complement-fixation by the Q-fever antigen as suggested by Stoker *et al* (1955). The complement-fixing antigens used were: Q-fever-Nine Mile strain, rickettsialpox and psittacosis produced by Lederle, and a murine-epidemic typhus antigen produced by Markham Laboratories.

In Phase II of the study, two small areas of hill-dipterocarp forest—designated Intensive Study Areas I and II—were investigated over a 7-month period to determine which animals are involved in a forest Q-fever cycle. These study areas, the trapping patterns,

11. Domiciliated animals are those which occupy man-made niches without encouragement, as opposed to domestic animals which have been encouraged to live in man-made niches and are directly under man's control, e.g., pets and livestock (Audy 1958).

and the animals collected are described in detail in Part 2 of this series.

RESULTS

Phase I. Q-fever antibody was detected in 0.8% ($\frac{11}{1425}$) of the small mammal serum samples that were not anticomplementary (Table 18). The positive animals were 1 *Rattus muelleri* collected in hill dipterocarp forest, 1 *R. r. diardi* from an agricultural area at the base of Gunong Jerai in NW Malaya, and 10 *Tupaia glis* (7 from Pulau Aur off SE coast of Malaya, 1 from fresh water swamp forest, and 2 from hill dipterocarp forest). None of the Q-fever positive animals had antibody to murine-epidemic typhus or psittacosis. Neither the *R. muelleri* nor the *R. r. diardi* had antibody to rickettsialpox antigen, but eight of the nine *T. glis* did have rickettsialpox antibody at titers equal to or greater than their Q-fever titers.

Table 18. Occurrence of Q-fever complement-fixing antibody in small wild mammals of Malaya.*

Species	Mountain Oak Forest	Upper Dipterocarp Forest	Hill Dipterocarp Forest	Lowland Dipterocarp Forest	Secondary Forest Scrub	Freshwater Swamp Forest	Mangrove Swamp Forest	Islands	Estates	Agricultural Land	Urban Areas	Total	Percent
Bandicota indica										0/1**		0/1	
Callosciurus caniceps			0/3		0/1					0/3		0/7	
C. hippurus			0/1									0/1	
C. nigrovitatus			0/12	0/5	0/1							0/18	
C. notatus	0/17		0/28	0/8	0/2	0/71	0/17	0/2	0/8			0/153	
C. prevosti						0/3						0/3	
C. tenuis				0/2		0/2						0/4	
Chiropodomys gliroides			0/4									0/4	
Lariscus insignis				0/1								0/1	
Mus musculus											0/18	0/18	
Rattus annandalei					0/2	0/17						0/19	
R. bowersi			0/15	0/3	0/7							0/25	
R. canus			0/8									0/8	
R. cremoriventer	0/10		0/7	0/2	0/1							0/20	
R. edwardsi	0/3	0/1										0/4	
R. exulans					0/8	0/2	0/1	0/3	0/1	0/19	0/9	0/43	
R. muelleri			1/44	0/4	0/12							1/60	1.7
R. norvegicus											0/38	0/38	
R. rajah	0/14	0/8	0/12	0/15	0/2							0/51	
R. rattus argentiventer										0/29	0/3	0/32	
R. r. diardi							0/1		0/32	1/66	0/135	1/234	0.4
R. r. jalorensis					0/24		0/42	0/2	0/82	0/29		0/179	
R. r. roa								0/63				0/63	
R. sabanus			0/50	0/27	0/5							0/82	
R. surifer	0/11	0/4	0/4					0/54				0/73	
R. whiteheadi	0/25	0/3	0/6	0/18	0/18	0/9						0/79	
Rhinosciurus laticaudatus			0/2			0/3						0/5	
Echinosorex gymnurus			0/6	0/3	0/1	0/2	0/1					0/13	
Hylomys suillus	0/1		0/2									0/3	
Suncus murinus										0/1	0/92	0/93	
Tupaia glis	0/25	0/6	2/10	0/19		1/1+		7/27				10/88	11.4
T. minor			0/2	0/1								0/3	
Total	0/106	0/22	3/216	0/108	0/84	1/110	0/62	7/151	0/123	1/148	0/295	12/1425	
Percent			1.4			0.9		4.6		0.7			0.8

* Samples fixing complement at a serum dilution of 1/4 or higher are considered positive for Q-fever antibody. Only those serum samples with no significant anticomplementary activity are included.

** The ratio of number of samples fixing complement at a serum dilution of 1/4 or higher to the total number tested.

\+ This animal also had CF antibody to rickettsialpox antigen.

Only one of these samples, however, also reacted to Rocky Mountain spotted fever antigen and none reacted to murine-epidemic typhus group antigen. One of the *T. glis* from Pulau Aur had a Q-fever titer of $\frac{1}{16}$ in the absence of antibody to any other antigen tested.

None of the 53 goat or 322 pig serum samples tested contained antibody against *C. burneti*. Only 4 of the 435 cattle serum samples were positive—3 at a titer of $\frac{1}{4}$ and 1 at $\frac{1}{8}$.

None of the 44 bird samples tested had demonstrable Q-fever antibody at a titer of $\frac{1}{8}$, the lowest level tested. However, there is some evidence that CF antibodies are poorly produced in birds (Shestochenko 1960), and this may not be a very reliable measure of Q-fever in this case.

One monkey, *Presbytis cristatus*, had an antibody titer of $\frac{1}{4}$. None of the other mammals tested possessed demonstrable antibody (Table 19).

Six of the 216 human serum samples were positive at low titers—5 at $\frac{1}{4}$ and 1 at $\frac{1}{8}$. One of the positive reactors was an aborigine, and the other five were soldiers who often are required to go into the forest on exercises.

Presumptive isolation of *Coxiella burneti* was made from one of the 433 pools of tissues from 1,849 wild animals. The positive pool contained tissues from five *Rattus sabanus* collected from hill dipterocarp forest in the Ulu Gombak Forest Reserve. This strain was carried through several egg passages, each of which caused a mild disease in guinea pigs with minimal or no febrile response, but did produce rises in antibody titer to $\frac{1}{256}$, 4 weeks after inoculation.

No *C. burneti* was recovered from any of the 33 pools of 1,175 ticks collected and processed during the survey phase of this study (see Table 3).

Phase II. Q-fever CF antibody was demonstrated in one *R. sabanus* and four *T. glis* in Study Area I (Table 20). Three of the four *T. glis* also reacted to rickettsialpox antigen at higher titer than to Q-fever antigen. The *R. sabanus* had no antibody to any other rickettsial antigen tested.

In Study Area II, Q-fever CF antibody was demonstrated in one *R. sabanus* and two *R. surifer*, each captured once only (Table 20). None of these animals had antibody to any other antigen tested.

In both study areas, a total of 675 ticks was collected. The predominant tick was *Dermacentor auratus* (306 collected). *Ixodes granulatus* (195 collected) and one or more species of *Haemaphysalis*[12] (total of 171) occurred but were less common. *Am-*

Table 19. Q-fever complement-fixing antibody in monkeys, other large wild mammals and birds of Malaya.

Species	No. positive* / No. tested
Macaca irus	0/166
M. nemestrina	0/2
Presbytis cristatus	1/74
P. melalophos	0/4
Manis javanica	0/1
Cynocephalus variegatus	0/1
Pteropus hypomelanus	0/5
Tragulus javanicus	0/36
Sus scrofa	0/1
Birds**	0/44
Total	1/334

* Samples fixing complement at a serum dilution of 1/4 or higher are considered to be positive for Q-fever antibody. Only those with no significant anticomplementary activity are included.

** The 19 species of birds included here are listed in Table 8.

Table 20. Q-fever complement-fixing antibody in animals collected in Intensive Study Areas I and II.*

Species	Study Area I	Study Area II
Callosciurus caniceps		0/3**
C. nigrovitatus		0/1
C. notatus		0/1
C. tenuis		0/5
Chiropodomys gliroides		0/3
Rattus annandalei	0/5	0/1
R. bowersi	0/6	
R. canus	0/1	
R. cremoriventer		0/5
R. edwardsi	0/7	0/2
R. muelleri	0/24	0/5
R. rajah	0/2	0/17
R. r. jalorensis		0/1
R. sabanus	1/179	1/52
R. surifer	0/3	2/24
R. whiteheadi	0/5	0/7
Rhinosciurus laticaudatus	0/1	
Echinosorex gymnurus	0/2	
Hylomys suillus	0/5	0/1
Tupaia glis	4/19+	
T. minor		0/1
Total	5/259	3/129

* Anticomplementary serum samples are not included.

** Ratio of the number of samples fixing complement at a serum dilution of 1/4 or higher to the total number tested.

+ All the Q-fever positive *T. glis* also had antibody to tick typhus. The Q-fever titers ranged from 1/4 to 1/8; the tick typhus 1/2 to 1/64.

12. Probably *H. papuana* Thorell and *H. semernis* according to Nadchatram (1965).

blyomma ticks were rare, only three being collected over a period of 7 months (Table 18). The hosts with the most ticks per animal were *R. muelleri*, *R. rajah*, *R. sabanus*, *R. annandalei*, *R. bowersi* and *R. edwardsi*. *Coxiella burneti* was not isolated from any of the 29 tick pools, each of which contained from 2–60 ticks.

DISCUSSION

Since the initial isolation and identification of *Coxiella burneti* in Australia three decades ago (Derrick 1937), its world-wide distribution has become apparent. Q-fever as a human disease is most commonly found in temperate or subtropical areas, but *C. burneti* probably occurs wherever there are rodents and ticks. The prevalence of Q-fever in the cooler regions of the world is due to the concentration of cattle and sheep in these zones. The close association of human Q-fever with domestic animals and their products in the United States and other temperate countries is well known (see, for example, Smadel 1959). Even in tropical environments, Q-fever in man usually is associated with domestic animals, a high percentage of which may be infected (Giroud 1951; Jadin 1951). In many tropical areas, however, where cattle and sheep are few and dairy products are not highly regarded as food, Q-fever in man is rare but not absent.

The conclusion drawn by Le Gac *et al* (1952) that *C. burneti* is not a tropical forest dweller is certainly open to question. It was based on the results of a skin test survey in which reaction to a Q-fever skin test antigen was shown by savannah-dwelling African natives, but not by equatorial forest pygmies. Q-fever is much more prevalent among savannah peoples who have contact with domestic ruminants and thereby contract Q-fever much more readily than forest dwellers. However, this evidence alone does not prove that *C. burneti* is absent from tropical forests.

In the tropics, as elsewhere, *C. burneti* can usually be found in wild rodents and their ticks, whether or not domestic animals are prevalent. Even where a domestic animal cycle of Q-fever occurs, another cycle in wild animals usually can be found. This is well demonstrated in Kenya, where a wide range of rodents and domestic animals is involved (Heisch 1960).

The prevalence of Q-fever antibody is low in domestic animals in Malaya, as determined in this and previous investigations (IMR 1951, 1954, 1955). Cattle, buffaloes, goats, and sheep apparently become infected occasionally, but there is little evidence for a distinct Q-fever cycle in domestic ruminants; and the pattern of human Q-fever does not tend to implicate domestic animals. Rather, human cases have usually occurred among people who spend considerable time in the forest. The only indication that Q-fever might have been contracted from association with domestic animals was the discovery of a butcher in Kelantan with a Q-fever CF titer of $_3^1{}_2^1$ (IMR 1951). This, however, is hardly proof enough to implicate domestic animals in maintenance and spread of Q-fever.

The evidence for a wild rodent cycle of Q-fever in Malaya, on the other hand, is overwhelming. Previous work at the IMR demonstrated that approximately 6% of over 400 rodents sampled possessed Q-fever antibodies, with the highest prevalence in the forest rats, *R. sabanus* and *R. rajah* (IMR 1960). This is considerably higher than was found in the 1962–1964 study. However, a much larger number of domiciliated and non-forest animals is included in this study than was the case in the 1960 investigation, and different areas were sampled. The two studies agree that *R. sabanus* and other forest rats are involved in maintaining *C. burneti* in nature. Rickettsiae were isolated from ticks collected from forest rats in the 1960 study, and *C. burneti* was isolated from tissues of *R. sabanus* in the present work, although it was not recovered from ticks.

The presence of Q-fever antibodies in house rats, *R. r. diardi*, is difficult to explain except by postulating that the infection was acquired indirectly from a forest animal via an infected tick. There is ample opportunity for domiciliated animals to become infested with forest ticks in scrub and secondary forests which are often adjacent to villages or towns. Domestic animals probably become infected in the same manner.

The high prevalence of Q-fever CF antibodies in *Tupaia glis* indicates that this primitive primate forms an important link in the forest cycle. However, 7 of the 10 positive animals in this study were collected on Pulau Aur, an island off the SE coast of the Malay Peninsula, where *T. glis* is one of three species of dominant wild animals.[13] Also, there is some question as to the specificity of the complement fixing antibody in this species. In most cases, complement was also fixed in the presence of rickettsialpox antigen. The role of these animals in Q-fever cannot be assessed properly until their reactions to experimental Q-fever and tick typhus infection are studied.

The primary natural cycle of Q-fever in Malaya resembles the forest-steppe cycles described by Karulin (1960) in that the circulation of rickettsiae is not limited to isolated populations of single species since there are no ticks restricted to a narrow host range. The *Ixodes*, *Haemaphysalis*, and *Dermacentor*

13. *Rattus surifer aoris* in the hill dipterocarp forest and *R. r. roa* in the kampongs are the other two dominant animals. *Tupaia glis* occurs mostly in the coconut groves, but is also found in the forest on this island (see Part I).

ticks of the tropical rain forest have wide host ranges among both small and large vertebrate populations. The present and earlier (IMR 1960) investigations in Malaya indicate that a number of large forest rats, *R. sabanus*, *R. rajah*, *R. surifer*, *R. muelleri*, are of prime importance in natural Q-fever cycles, but other species, such as *T. glis*, may also be involved. The participation of *Ixodes granulatus* and *Haemaphysalis* spp. (IMR 1960) is shown by their preference for these rats and the isolation of *C. burneti* from both species. Their efficiency in transmitting and maintaining the rickettsiae is not known.

Although the existence of natural cycles of *C. burneti* in forest animals has been demonstrated, the actual mechanism of transmission of the rickettsiae and its maintenance in ticks and rodents must await further study. The low virulence (for guinea pigs) of the strain of *C. burneti* isolated from *R. sabanus* also must be evaluated in the laboratory and confirmed by further isolations from forest animals. The low prevalence of CF antibody in domestic animals does not strongly suggest the presence of a secondary Q-fever cycle in these animals, but additional work is needed in this area too.

SUMMARY AND CONCLUSIONS

A serological survey of 1,425 small mammals and 334 larger wild mammals and birds collected from all the major ecosystems throughout Malaya demonstrated the presence of Q-fever CF antibody in 12 small mammals (*R. muelleri*, *R. rattus diardi*, and *T. glis*) and one monkey (*Presbytis cristatus*). A more intensive study of a Q-fever focus in hill dipterocarp forest uncovered additional sero-positive *R. sabanus*, *T. glis*, and *R. surifer*.

No Q-fever antibody was detected in pigs or goats, and less than 1% of over 400 cattle serum samples tested were positive. Six of 216 human serum samples tested had antibody to *C. burneti*. All six of the people from whom the positive samples were obtained were soldiers who spend time in the forest.

The only strain of *C. burneti* recovered from wild animal tissue samples came from *Rattus sabanus* collected in hill dipterocarp forest. None of the 62 pools of ticks inoculated into guinea pigs produced any evidence of the presence of *C. burneti*.

Analysis of these results and those of previous workers in Malaya indicates that the basic natural cycle of Q-fever exists in small wild mammals of the forest and their ectoparasitic ticks, primarily *Ixodes granulatus* and *Haemaphysalis* spp. Although domestic animals may become infected with *C. burneti*, there is no substantial evidence that they support a secondary Q-fever cycle independent of the cycle in wild animals.

PART 4. URBAN TYPHUS, WITH NOTES ON THE OCCURRENCE OF PSITTACOSIS ANTIBODY IN MONKEYS

Urban or murine (flea-borne) typhus has been known to exist in Malaya for many years, but it was long confused with tropical or scrub (mite-borne) typhus. Dyer *et al* (1931) and Mooser *et al* (1931) resolved the difficulty by conclusively demonstrating in North America the existence of an endemic infection of rats; when transmitted to man, this infection caused a typhus-like disease. Subsequently, Lewthwaite & Savoor (1936 a, b, c) isolated *Rickettsia mooseri* from rats and man in Malaya and showed that it is distinct from *R. tsutsugamushi*, *R. rickettsii* and *Coxiella burneti*.

Once the distinction between urban and scrub typhus was recognized in Malaya, attention was focused on the much more serious problem of scrub typhus. The primary interest in urban typhus was in its relation to plague. The incidence of urban typhus in Malayan towns constituted a useful indication of possible future human plague outbreaks because of the similarity in their modes of transmission. An increase in urban typhus cases would mean an increase in the rat and oriental rat-flea populations and consequently a potentially hazardous plague situation. An intensive investigation of thousands of rats in Kuala Lumpur in 1941 revealed no plague bacilli, but uncovered an active focus of urban typhus in the center of the city (IMR 1951). This occurred in a former plague area where human cases of urban typhus had appeared year after year for many years. Plague no longer occurs in Malaya, but urban typhus lingers on with cases occurring every year.

The present study was undertaken primarily as a corollary to studies of tick-borne rickettsioses in Malaya (see Parts 1, 2, 3), and to further investigate the possible involvement of wild forest animals as well as domiciliated and domestic animals in urban typhus ecology. An earlier study (IMR 1955) indicated that forest rats in Malaya are not involved, but work in Africa suggested that rodents not directly associated with man may be naturally infected with *R. mooseri* (Heisch & Harvey 1959; Heisch *et al* 1962). Also the isolation of *R. mooseri* from cattle ticks (*Boophilus australis*) in

India (Kalra & Rao 1949) suggested the implication of large domestic animals in urban typhus.

MATERIALS AND METHODS

The 1182 small wild mammals, 224 monkeys, 12 mouse deer, 39 birds, 471 domestic animals and 218 humans tested are part of those already described in Part 1. The collecting methods and the tissue, serum and tick processing methods are similar to those described in Parts 1 and 2, and will be discussed only when they differ. A group murine-epidemic typhus complement-fixing antigen produced by Markham Laboratory was used in the semi-micro complement-fixation test (Sever 1962) for all sera. A number of serum samples also was tested for psittacosis complement-fixing (CF) antibodies using an antigen produced by Lederle. All samples with a titer of 1: 4 or higher were retested and not considered positive unless at least two tests gave essentially the same titer. In addition, all samples were tested for tick typhus and Q-fever antibodies as already described (Parts 2 & 3).

RESULTS

The prevalence of urban-epidemic group CF antibody in 30 species of small mammals tested is shown in Table 21. Most of the animals containing typhus antibody were those closely associated with man in urban or other man-dominated habitats (estates and agricultural land). The greatest number of positive samples (12) came from *Rattus rattus diardi*, the house rat, collected from urban areas. One *R. exulans* and two *Suncus murinus*, both from urban environments, possessed typhus antibodies in low titer (Table 22). The specific areas from which these typhus positive animals came are shown in Table 23.

None of the 53 goat or 82 pig serum samples tested contained group typhus antibody; but 2 of the 336 cattle samples tested had antibody at titers of 1: 4 and

Table 22. Complement fixing antibody levels to murine-epidemic typhus group antigen in man-associated mammals in Malaya.

Species	No. Tested	Antibody Titer*		
		4	8	16
R. exulans	41	1		
R. rattus	271	9	2	3
S. murinus	85	1	1	
Total	397	11	3	3

* Reciprocal of highest serum dilution fixing complement in the presence of murine-epidemic typhus group antigen.

Table 21. Complement-fixing antibody to group murine-epidemic typhus antigen in small mammals collected from various ecosystems in Malaya.

Species	Climatic Climax Forests*	Secondary Forest	Freshwater Swamp Forest	Mangrove Swamp Forest	Estates, Agric. Land	Urban Areas	Total	% Positive
Small Forest Animals**	0/435+	0/22	0/22				0/479	
Callosciurus notatus			0/7	0/25	0/2		0/34	
Mus musculus						0/16	0/16	
Rattus exulans		0/8	0/2	0/1	0/22	1/8	1/41	2.4
R. r. argentiventer					0/28	0/3	0/31	
R. r. diardi				0/1	2/84	12/123	14/208	6.7
R. r. jalorensis		0/24		0/83	0/63		0/170	
R. r. roa†					0/63		0/63	
R. whiteheadi		0/18	0/9				0/27	
Echinosorex gymnurus		0/1	0/2	0/1			0/4	
Suncus murinus					0/1	2/84	2/85	2.3
Manis javanicus						0/1	0/1	
Tupaia glis					1/23‡		1/23	
Total	0/435	0/73	0/42	0/111	2/286	15/235	18/1182	
Percent					0.7	6.4		1.5

* Includes: mountain oak, upper dipterocarp, hill dipterocarp and lowland dipterocarp forest ecosystems.

** Forest animals included: *Rattus annandalei, R. bowersi, R. canus, R. r. cremoriventer, R. edwardsi, R. muelleri, R. rajah, R. surifer, R. whiteheadi, Callosciurus notatus, C. nigrovitatus, C. tenuis, C. caniceps, Rhinosciurus laticaudatus, Lariscus insignis, Chiropodomys gliroides, Tupaia glis, T. minor, Echinosorex gymnurus, Hylomys suillus.*

\+ The numerator is the number of specimens fixing complement at a serum dilution of 1/4 or higher in the presence of murine-epidemic group antigen. The denominator is the total number tested.

† From coconut plantations on Pulau Aur.

‡ Also had an equal titer (1/8) to rickettsialpox antigen.

Table 23. Geographical distribution of species with demonstrable murine-epidemic typhus complement fixing antibody.*

Species	Paroi	Kuala Lumpur	Petaling Jaya	Klang	Batang Berjuntai	Total
Rattus exulans	0/1**	1/8				1/9
R. r. diardi	1/20	12/123		1/3	1/6	15/152
Suncus murinus	0/1	1/69	1/15			2/85
Total	1/22	14/200	1/15	1/3	1/6	18/246

* These cities and towns are located in the states of Selangor and Negri Sembilan in the West-Central part of the Malay Peninsula.

** The numerator is the number of samples fixing complement at a serum dilution of 1/4 or higher. The denominator is the total number tested.

Table 24. Mammals from which presumptive isolations of urban typhus rickettsiae were made in Malaya.

Species	Locality	Ecosystem
Rattus exulans	Kuala Lumpur, Selangor	Urban area
R. r. diardi	Kuala Lumpur, Selangor	Urban area
R. r. diardi	Paroi, Negri Sembilan	Agricultural land
R. r. diardi	Paroi, Negri Sembilan	Agricultural land
R. r. jalorensis	Rantau Panjang, Selangor	Nipa palm Mangrove swamp edge
Suncus murinus	Kuala Lumpur, Selangor	Urban area

Table 25. Psittacosis complement-fixing antibodies in Monkeys.

Species	Antibody titer*			
	Neg.	4	8	16
Macaca irus	132	15	8	2
M. melalophos	2			
M. nemestrina	2			
Presbytis cristatus	69	2		
Total	205	17	8	2

* Reciprocal of highest serum dilution fixing complement in the presence of psittacosis CF antigen.

1: 8 respectively. Only one of the 218 human serum samples was positive and that at a titer of 1: 4.

None of the 12 mouse deer (*Tragulus javanica*) nor 224 monkeys (*Macaca irus, M. nemestrina, Presbytis cristatus*) possessed antibody. One pied fan-tailed flycatcher had an antibody titer of 1: 8, but none of the other 38 birds of various species tested was positive.

Presumptive isolations of typhus rickettsiae were made from 6 pools of tissue from 4 species of mammals (Table 24). Three of the positive pools contained tissues of animals collected in rural[14] habitats, and three contained tissues of animals collected in the city (Kuala Lumpur).

No attempt was made to test fleas and mites for group typhus rickettsiae. Ticks occurred only rarely on domiciliated animals but one batch from a variety of sources was pooled and inoculated into guinea pigs. Unfortunately this pool contained a miscellaneous lot of ticks: 33 *Haemaphysalis centropi* from "ground birds" collected from secondary forest-scrub near Kuala Lumpur, 61 *Amblyomma helvolum* from *Varanus salvator* from agricultural areas near Malacca, 2 *Amblyomma* spp. from *R. r. jalorensis* collected from Pulau Pangkor, 1 *Amblyomma* sp. from *R. bowersi* from Kedah Peak, and 19 *A. javanica* from *Manis javanicus* collected in Kuala Lumpur. Although the original tick pool produced complement-fixing antibody titers of greater than 1: 16 in both guinea pigs into which it was inoculated, 4 of the 5 individual tick pools making up the original positive pool caused no antibody response upon reinoculation into additional guinea pigs. The remaining pool (*A. javanica* from *M. javanicus*) was heavily contaminated with a *Salmonella*-like bacillus that killed the guinea pigs in 5 days and masked whatever *R. mooseri* might have been there. The addition of higher concentrations of antibiotics to the inoculum in a repeat test failed to control the bacterial infection and inoculated guinea pigs again died in 5 days. No samples remained for further retesting.

No psittacosis antibody was detected in 66 small mammals from a variety of habitats. However, 25 of

14. The term "rural" is used here in its civil law definition: land adapted and used for agricultural or pastoral purposes as opposed to "urban" which refers to cities or towns where the land is used primarily for human dwelling.

132 *Macaca irus* and 2 of 69 *P. cristatus* were found with antibody in titers from 1:4 to 1:16 (Table 25).

DISCUSSION

In the serological survey reported here, an urban-epidemic typhus group CF antigen was used and the specific type of antibody was not determined; nor have the rickettsial isolates been identified to species. However, in the apparent absence of human body lice and any previously reported evidence of epidemic typhus in Malaya, one may assume that the organism involved here is *R. mooseri* and not *R. prowazeki*. Furthermore, *R. prowazeki* has never been isolated from house rats nor any other rodents, whereas the natural host of *R. mooseri* is the house rat.

Antibody against group typhus antigen was found most often in animals which live in close association with man in urban or rural areas. Three of the 6 isolates were from *R. r. diardi*, the Malaysian house rat. The presence of CF antibody and the presumptive isolation of rickettsiae from the house shrew, *S. murinus*, indicates that it also may become infected with murine typhus. This is the first report known of the involvement of *S. murinus* in urban typhus ecology.

The local distribution of murine typhus in and around urban centers in Malaya is similar to that in other endemic areas. The lack of definitive evidence that forest mammals are involved agrees with the results of Gear (1954) in Africa, who showed that veld rodents were not infected nor did they possess CF antibody. On the other hand, in the Rift Valley of Africa it has been shown that wild rodents of several species possess low level CF antibodies to murine typhus (Heisch & Harvey 1959; Heisch *et al* 1962).

The isolation of *R. mooseri* from *R. r. jalorensis* collected in a rural area in Malaya shows that animals other than house rats and house shrews may become infected. This particular *R. r. jalorensis* was collected from an edge habitat between an edaphic climax mangrove swamp forest and a nipa palm-coconut plantation. Several houses were located nearby and it may have contracted the infection from a house rat.

The sero-positive bird collected from secondary forest probably also contracted its typhus infection from domiciliated animals since this bird is particularly common in gardens and orchards (Madoc 1956).

The evidence thus far confirms the dependence of the murine typhus cycle on house rats in Malayan cities and towns, but the occurrence of murine typhus in *Rattus rattus* in non-urban areas indicates that rural foci of this disease also occur.

The significance of the rickettsial isolation from the pool of ticks collected from various sources is unknown since it could not be determined from which tick the isolation was derived. The pangolin (*M. javanicus*) from which some of the ticks in the pool were obtained was collected on the outskirts of Kuala Lumpur. The pangolin itself was not infected nor did it possess typhus antibodies. In the absence of further data, the validity of this isolation must remain suspect. The isolation of *R. mooseri* from a tick in India (Kalra & Rao 1949) and *R. prowazeki* from numerous ticks in North Africa (Reiss-Gutfreund 1956, 1961) has been reported, but the role of ticks in the ecology of both urban and epidemic typhus must be more critically studied.

The presence of typhus antibody in cattle is also difficult to interpret, and its detection in a few animals in low titer (1:4 and 1:8) may not represent specific urban typhus antibody. However, Reiss-Gutfreund (1956, 1961) also found urban (and epidemic) typhus antibody by the microagglutination test in domestic animals in North Africa. In Malaya domestic animals (cattle and goats) commonly occur in towns and cities where they could be exposed to murine typhus infection, but the actual mechanism of transmission is not known.

The presence of psittacosis (ornithosis) in Malaya was demonstrated in 1959 when complement-fixing antibody was found in three domestic pigeons and a strain of the organism was presumptively isolated from pigeon droppings (IMR 1959, 1960). The brief serological survey for psittacosis antibody in wild animals reported here indicates a high level of positive reactors in the monkey population. However, in the absence of definitive isolation of the organism from monkeys and of information on the reaction of these animals to infection with psittacosis, the results are again difficult to interpret. The specificity of the antibody reactions has not been determined, but the positive animals reacted only to psittacosis and not to murine-epidemic typhus, Q-fever nor rickettsialpox complement-fixing antigens.

The possible involvement elsewhere of wild mammals in psittacosis ecology is suggested by the isolation of a member of this group of organisms from opossums in South America (Roca-Garcia 1949) and by the demonstration of psittacosis antibodies in a variety of wild mammals in the U.S. (Stoenner *et al* 1959; Sidwell *et al* 1964). In the latter study the organism was isolated from a domestic pigeon, but could not be demonstrated in any of the wild animals tested.

SUMMARY

In 1182 small wild mammals of 30 species tested, complement-fixing antibody to group urban-epidemic typhus antigen was detected only in those living in close association with man. The Malayan house rat, *Rattus rattus diardi*, was most often implicated both serologically and by presumptive isolation of the or-

ganism. Other domiciliated species, *R. exulans*, *R. r. jalorensis* and *Suncus murinus* also possessed either antibody or rickettsiae. Presumptive isolation of typhus organisms was made from tissues of 3 *R. r. diardi*, 1 *R. exulans*, 1 *R. r. jalorensis*, 1 *S. murinus*, and one pool composed of a variety of ticks from various sources.

Low-level antibody was detected in two cattle, but not in goats or pigs. Except for one sero-positive bird from secondary forest-scrub, the only evidence of murine typhus activity occurred in domiciliated animals living in urban or rural areas in close association with man.

Psittacosis CF antibody was demonstrated in 25 of 132 *Macaca irus* and 2 of 69 *Presbytis cristatus*, but in none of 66 small wild mammals tested.

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