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ATTEMPTS TO IDENTIFY WILDLIFE RESERVOIRS OF RABIES *) IN INDONESIA

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Penyakit rabies telah dikenal di Indonesia sejak tahun 1889, dan mengingat tidak adanya data yang diteliti mengenai penderita rabies, maka perlu dilakukan suatu penelitian. Penelitian dilakukan oleh NAMRU-2 bersama Departemen Kesehatan dari tahun 1970-1972 untuk menentukan pengaruh penyakit-penyakit Zoonotic yang endemic pada penduduk yang tidak immune yang sering berpindah ke hutan atau tempat yang tidak ada penduduknya. Penelitian di lakukan pada beberapa daerah pegunungan Jawa Barat, Jawa Timur, Lampung, Maluku, Kalimantan Tengah, Timor dan Sulawesi Tengah.

Hasil penelitian menunjukkan bahwa dari 328 binatang yang diperoleh dari 28 berbagai daerah di Indonesia setelah diadakan pemeriksaan dengan metode fluorecent antibody technique (fat) dan inokuler pada tikus putih ternyata tidak ditemukan virus rabies.

Binatang-binatang yang ditemukan tersebut dan kebiasaan hidupnya di lukiskan secara terperinci pada hasil penelitian ini.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care and handled in accordance with the principles outlined in the "Guide for the Care and Use of Laboratory Animals", U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

Rabies has been known in Indonesia at least since 1889 and, anecdotally, its origin is shrouded in antiquity. The emphasis placed on rabies in domestic animals and humans has obscured the possible role of wildlife animals in the dissemination of rabies throughout much of the archipelago. Accurate statistics on total cases of rabies is not available, but Biofarma (formerly the Pasteur Institute) in Bandung verifies its presence based on the relative rate of positive specimens detected during the past several years, as follows: 51% (1965); 67% (1966); 52% (1968); 35% (1969) and 52% (1970) (M.S. Nasution, personal communication).

One of the measures proposed for control of the disease has been to rule out the possibility of wildlife rabies in mammals including wild cats, bats, wild hogs, rodents, etc. (Ressang, 1960). Such an investigation was undertaken as part of the "developmental area study", a medical research project undertaken by the Indonesian Ministry of Health and the U.S. Naval Medical Research Unit No. 2 (NAMRU-2) to determine the influence of endemic zoonotic diseases on non-immune humans moving into previously forested and uninhabited areas. The data presented here were compiled from surveys made at different location between 1970-

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1972 in an effort to gain knowledge of the prevalence of rabies in wildlife populations from the areas described.

MATERIALS AND METHODS

Descriptions of localities Sampled

During the course of this study, 28 areas, representative of seven kinds of habitats, were surveyed as follows (Fig.):

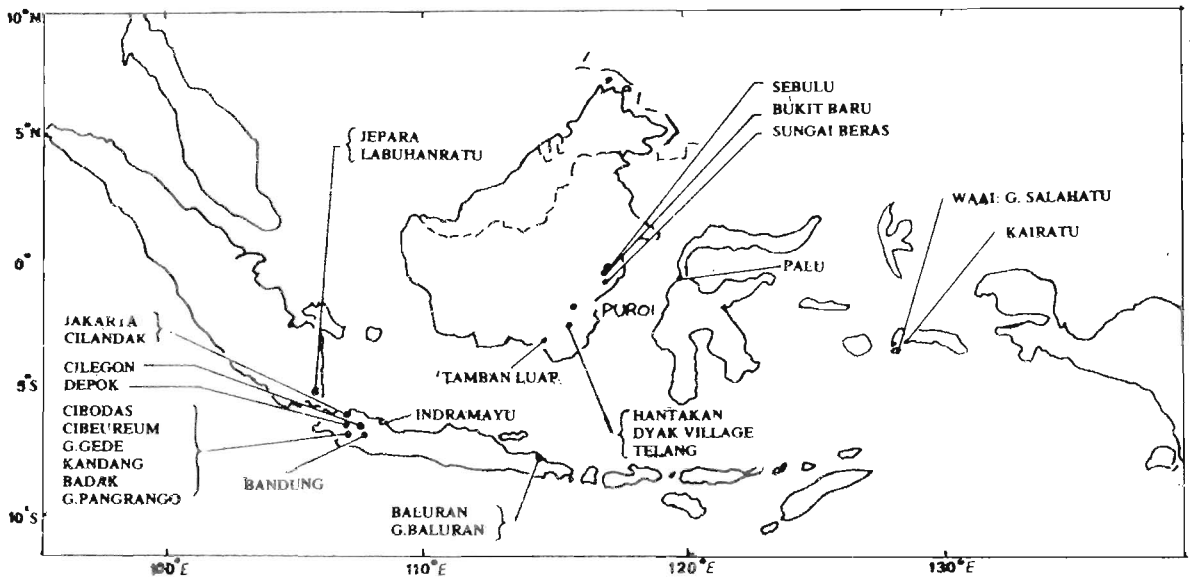

Fig. Map of Indonesia showing locations of surveyed sites.

valley between Mts. Pangerango and Gede. The summits of Mts. Gede (6°47' S, 106° 59'E, 2600 m) and Pangerango (6°47' S, 106° 58' E, 3005 m), also protected, are covered with scrub oak. There is a volcanic crater at the top of Mt. Gede.

Bandung (6°54' S. 107°37' E, 700m) is a large city on a plateau ringed with mountains, about 200 km southeast of Jakarta. Ciloto (6°43' S, 106°00'E, 1350 m) is an area of vacation cabins east of a high (1400 m) mountain pass traversed by the main highway from Jakarta to Bandung.

West Java lowlands; Mammals were collected on the broad flat rice-growing northern coastal plains of West Java which surround the capital city of Jakarta (6°10' S, 106°45' E, 10m), which has a population of over 5 million . Cilandak (6°16' S, 106°47' E, 25 m) is a southern suburb of Jakarta. Depok (6°24' S, 106°50' E, 65 m) is a village turther south in a densely populated area. The smaller cities of Indramayu (6°19' S, 108°19' E, 5 m) and Cilegon (6°32' S, 107°25' E, 110 m) are to the east of Jakarta. Cilegon is at the base of foothills leading to the Bandung plain.

Highlands of West Java. Cibodas (6 45'S, 107° 00 E, 1350 m) is a world-famous botanical garden, with many introduced fluorishing exotic trees and shrubs, situated on the slopes of Mt. Gede, southeast of the capital city of Jakarta. A rainforest above Cibodas is part of a nature preserve which includes a waterfall at Cibeureum (6°45'S, 10 10°00 E, 1650 m), and a clearing, Kandang Badak (6°45' S, 106°59' E, 2425 m),in a high

East Java: Localities visited in East Java were in a nature preserve at the far eastern most tip of the island. Baluran (7°51' S, 114° 27' E. 50 m) is a low savannah-like plain from which Mt. Baluran (7°50'S, 114°22' E, 620 M) rises. The mountain has relatively undisturbed deciduous primary forest.

Lampung, South Sumatra: Lampung is the southernmost province of Sumatra.

Jepara (5°13' S, 105°42' E, 40 m) and Labuhan Ratu (5°10' S, 105°42' E, 40 m) are in low swamp of a vast flat alluvial plain. This is a potentially rice-growing are being settled by "transmigrants" from less fertile areas in Central and East Java.

Central Maluku: All Maluku Islands are east of Wallace's Line. Waai, a small seaport, (3°34' S, 128°19' E, 2 m) and Mt. Salahutu (3°34' S, 128°17' E, 250 m) are on Ambon Island, near the provincial capital of Ambon. Areas studied on the lower reaches of Mt. Salahatu were heavily cut over to accommodate clove plantations. Kairatu (3° 16' s, 128°24' E, 70 m) is a port on the south coast of adjacent Ceram Island; some trapping was done 10 Km inland, in disturbed rainforest.

South, Central and East Kalimantan : Localities studied in Kalimantan were on the eastern edge of this, the third largest island in the world. Tamban Luar (3°14' S, 114°22' E, 1 m), a tiny settlement in virgin tidal swamp forest, is the only locality acutally within the borders of Central Kalimantan Province. Hantakan (2°38' S, 115°27' E, 50 m), Teland (2°30' S, 115°24' E, 20 m) Dyak Village (2°42' S, 115°28' E, 400 m), and Puroi (1°53' S, 115°41' E, 100 m) of S. Kalimantan Province are small villages situated along the main north-south highway of Indonesian Kalimantan (Borneo). All are in heavily cut-over rain forest. Bukit Biru (00° 30' S, 116°58'E, 208 m) is a transmigrant village in East Kalimantan Province while Sungai Beras (00°55'S, 117°00'E, 70 m) and Sebulu (00° 16'S, 117° 00'E, 100m) are settlements in virgin rainforest presently being harvested by timber companies.

Central Sulawesi.The only mammals trapped in Central Sulawesi (Celebes) during the present study was in the provincial capital of Palu (00° 52'S, 119° 54'E, 5 m) a flat dry coastal city surrounded by coconut plantations.

Field Procedures

Captures were made in the surveyed areas between 1970-1972 using small Sherman, NAMRU-2 box, or National live traps. Animals were anesthetized with chloroform, exsanguinated by cardiac puncture, skinned, and skulls freed of extraneous tissue. Brain material was extruded through the foramen magnum into sterile petri dishes by forceful injection of sterile physiologic saline into the skull casing using a syringe and 18 gauge needle. Barins were placed into 3-5 ml of sterile glycerol-saline (50-50 V/V) in 2 dram vials. The specimens were returned to the laboratory usually within two weeks and stored at -70°C until examination.

Mouse Inoculation

Twenty-one day old outbred Swiss white mice of both sexes were distributed in groups of five in escape-proof glass jars. Each inoculum was prepared by grinding approximately 1 gm of brain tissue in 10 ml phenol broth lactalbumin hydrolysate (PBLH) diluent. In most cases this inoculum included the hippocampus, cerebrum, cerebellum, cortex and brainstem. Mice were anesthetized with ether and inoculated intra-cerebrally with 02 ml of the suspension using a tuberculin syringe with 26 gauge needle. Inoculated mice were observed daily for a period of thirty days (Koprowski, 1973). Those that died three days post-inoculation or later were examined for the presence of rabies virus using the fluorescent antibody technique.

Fluorescent Antibody Technique

The fluorescent antibody technique (FAT) was performed according to the method of Goldwasser et al (1959). The conjugate (Sylvana Co., Milburn, N.J.) was a fluoresce-in-labeled anti-rabies globulin. The dessicated product was reconstituted, absorbed with mouse brain powder and titered to a working dilution.

There has been some criticism of the FAT when examining glycerolated material since there is a presumable quenching of fluorescence (McQueen et al., 1960). Additional evidence seems to indicate taht quenching is directly related to acetone fixation of glycerol treated material (Andrulonis and Debbie, 1975). Use of FAT according to described methodology should maintain efficiency with either type of specimen (Goldwasser et al., 1959). It is significant that

glycerol addition does not detract from the suitability of these specimens for mouse inoculation studies, which, although more time consuming, is still considered the most reliable single method available for detection of rabies (Tierkel, 1973).

RESULTS AND DISCUSSION

The table provides the total number of animals examined according to locality. Of the 328 animals tested by FAT and mouse inoculation, none were found to harbor rabies virus. Tha habitats of these animals and potential impact are described as follows

COMMENSALS.

Many of the mammals examined are usually found in or near human habitation. *Suncus murinus*, the common house shrew, *Rattus diardii*, the brown rat, *Rattus norvegicus*, the Norway rat, and *Mus musculus*, the house mouse, are invariably commensal throughout their widespread distribution in Southeast Asia. With the possible exception of *R. diardii* all are probably introduced to Indonesia. Although

Animals Examined for Rabies in Various Localities of Indonesia (cont'd)

	Locality and Number Collected[1]
Order Insectivora	
Family Erinaceidae	
Hylomys suillis, lesse gymnure	I(7)
Family Soricidae	
Suncus murinus,musk shrew	VI(1), XVII(1), XX(1), XXI(1)
Family Tupaiidae	
Tupaia tana, tree shrew	XXI(2)
Tupaia minor, lesser tree shrew	XXI(1)
Order Chiroptera	
Famili Pteropodidae	
Chironax melanocephalus, black-capped fruit bat	I(2)
Cynopterys brachyotis, common short-nosed fruit bat	I(10), VI(2), XX(2), XXI(7), XXII(1) XXIV(13), XXV(3), XXVI(1)
Cynopterus horsfieldi, short-nosed fruit bat	I(2), XXI(2), XXIV(1)
Cynopterus sphinx, short-nosed fuit bat	I(2), VI(3)
Pteropus vampyrus, large flying fox.	VI(1)
Rousettus amplexicaudatus, rousette bat	XVII(2), XIX(3)
Eonycteris spelaea, long-tongued bat	I(1), XXI(4), XXV(3)
Marcroglossus minimus, nectar feeding bat	XXI(1)
Eonycteris major	XXI(1)
Penthetor lucasi	XXI(1), XXVI(1)
Dobsonia moluccensis	XVII(1), XIX(4)
Syconycteris crassa	XVII(2), XVIII(1), XIX(30)
Nyctimene cephalotes	XVIII(1), XIX(3)
Family Emballonuridae	
Emballnura monticola, sheath-tailed bat	XXI(1)

Animals Examined for Rabies in Various Localities of Indonesia (cont'd)

	Locality and Number Collected.†
Family Rhinolophidae	
Taphozous saccolaimus, tomb bat	I(2)
Rhinolophus borneensis	XXIII(1)
Hipposideros diadema, larga malay leaf-nosed bat	XXI(1)
Family Vespertilionidae	
Pipistrellus circumdatus	X(1)
Scotophilus temminicki	VII(12)
Myotis horsfieldi	XXI(2)
Order Primates	
Family Lorisidae	
Nycticebus coucang, slow loris	IV(2)
Family Cercopithecidae	
Macaca fascicularis, crab eating macaque	VIII(1)
Presbytis cristatus, silvered leaf monkey	XXI(1)
Masalis larvatus, long nosed monkey	XXIV(1)
Macaca tonkeana	XXVIII(1)
Other Rodentia	
Family Sciuridae	
Callosciurus notatus, plantain tree Squirrel	XIV(1), XXI(6), XXIV(1), XXVI(1)
Family Muridae	
Mus musculus, house mouse	VI(1), XVIII(2)
Mus [Mycteromys] vulcani	I(1)
Rattus argentiventer, ricefield rat	XXI(2)
Rattus bartelsii, rat	I(35), II(2), XII(2)
Rattus cremoriventer, dark tailed spring rat	I(2), XXI(3), XXIII(1)
Rattus diardii, Malaysian house rat	I(9), III(2), V(1), VI(1), XIV(3), XV(6), XX(2), XXI(1)
Rattus fulvescens', chestnut rat	I(1)
Rattus exulans, little pacific rat	I(23), II(1), XI(2), XVII(1), XX(1), XXI(1), XXII(1), XXIV(3), XXVII(4)
Rattus niviventer, white-bellied rat	I(6), XIII(1)
Rattus norvegicus, norway rat	VI(5)
Rattus rajah, brown spring rat.	XVI(1)
Rattus sabanus, noisy rat	I(1), XXVI(2), XXVII(2)
Rattus surifer, red spring rat	XXII(2), XXVI(2), XXVII(1)
Rattus tiomanicus, Malaysian field rat	XV(2), XXI(2)
Rattus mulleri	XXIII(3), XXVII(1)
Rattus whiteheadi	XXII(2), XXVII(1)
Rattus amboinensis	XVII(2), XVIII(2), XIX(1)
Rattus rattus	XXI(1), XXIV(1), XXVI(1)
Rattus sp.	I(3)

Animals Examined for Rabies in Various Localities of Indonesia (cont'd)

	Locality and Number Collected.[1]
Order Carnivora	
Family Mustelidae	
Melogale orientalis, ferret badger	II(1)
Mydaus javanensis, teledar	XXI(1)
Family Viverridae	
Viverricula indica, small civet	IX(1)
Paradoxurus hermaphroditus, palm civet	XVI(1), XXIII(1)
Hemigalus derbyanus	XXVI(1)

Key : Roman numeral represent locality. Number in parenthesis represent number of specimens.

Areas-Java : Cibodas-I;Cibeureum-II, Ciloto-II, Cilegon-IV; Cilandak-V, Jakarta-VI, Depok-VII;Bandung-VIII;Indramayu-IX;Kandang Badak-X;G.Pangrango-XI; G.Gede-XII;G.Baluran-XIII; Baluran-XIV.

Sumatra : Jepara(Lampung)-XV;Labuhanratu(Lampung)-XVI.

Ambon : Waai (Ambon)-XVII;G.Salahutu (Ambon)-XVIII:Kairatu (Ceram)-XIX.

Kalimantan : Telang-XX;Hantakan-XXI;Puroi-XXII:Dyak-XXIII;Tamban Luar-XXIV; Bukit Biru-XXV;Sebulu-XXVI;Sungai Beras-XXVII.

Sulawesi : Palu-XXVIII

not commensals, many bats examined during this study were caught near houses, or in gardens; species were within the genera *Cynopterus* short-nosed fruit bats, and *Rousettus*, Rousette bats, as well as *Macroglossus minimus*, the long-tongued nectar feeding bat. The vespertilionid bat, *Scotophi lus temminckii*. Commonly roosts in thatched roofs.

SYLVATIC SPECIES.

Some of the mammals examined are, in our experience, almost always found in primary forest. unassociated with human activity. Such mammals include the insectivore *Hylomys suillus*, the rodents *Mus vulcani*, *Rattus bartelsii*, *R. cremoriventer*, *R. fulvescens*, *R. niviventer*, *R. sabanus*, *R. rajah*, *R. surifer*, and *R. mulleri*, and the carnivore *Hemigalus derbyanus*

POSSIBLE "TRANSITION" SPECIES.

Of mammals not described above, many including the tree shrews *Tupaia tana* and *T.minor*, most bats, carnivores such as the skunk *Mydaus javanensis*, the ferret-badger *Melogale orientalis*, the civet *Viverricula indica*, and primates of the genera *Macaca* and *Presbytis*, are often trapped near areas of human activity. Little is known of the habits and ecology of these mammals, so that, for example, catching certain bats from mist nets set near villages may be happenstance, or may indicate that bats were purposefully foraging in fields or gardens. Similarly, macaques may raid gardens out of desperation or preferentially.

In experience certain Indonesian mammals normally occupy habitats created by man, and thus may serve as "transition"

species, which could transfer pathogens from strictly sylvatic mammals to those closely associated with man -- or vice-versa. In the case of rodents, such species include the little Pacific rat, *Rattus exulans*, which is absolutely ubiquitous, having been trapped inside the volcanic crater of Mt.Gede, as well as inside houses in cities, the brown rat, *Rattus rattus* (including *R. rattus amboinensis*), found in gardens and houses, and the rice-field rat *R. argentiventer*, and the field rat *R. tiomanicus*, which, as their names imply, are most commonly found in rice fields and scrub, respectively. The palm civet, *Paradoxurus hemaphroditus* and the squirrel, *Callosciurus notatus*, although sometimes trapped far from human habitation, are often pests of chicken-houses and coconut plantations, respectively.

Our knowledge of wildlife reservoirs of rabies in many countries is superficial. In the United States, the occurence of domestic rabies, primarily in dogs, prior to the 1950's completely prevailed over the wildlife form. Following the 1950's, there was an apparent reversal, so that wildlife rabies now constitutes a much larger proportion of the total number of reported cases. In retrospect and, in the face of successful animal vaccination programs, this appears to indicate that the domestic animal probably serves only as an incidental host in perpetuation of rabies among species in the sylvatic environment. The suspicion is that the sylvatic rabies situations has not changed dramatically over the years. Only because of the rapid decline of urban rabies, has the significance of wildlife rabies come more clearly into focus (McLean, 1970)

It is difficult to assess the relative importance of wildlife as a "silent partner" in the perpetuation of rabies in Indonesia. This study was associated primarily with surveys of proposed transmigrant areas (Van Peenen *et al.*, 1974) and there was no effort to evaluate the impact of wildlife on the incidence of rabies in domestic animals.

Rabies or rabies-like virus has been found elsewhere in many of the small mammals examined in this study (Sodja *et al.*, 1971, 1971a; Smith *et al.*, 1967, 1968). In the United States rodents account for a smallbut persistent segment of the total number of animals reported rabid each year. In the absence of endemic rabies among rodent, there has been no evidence to suggest that they contribute substantially to rabies epidemiology (Winkler, 1972)

Although this investigation was not an intense study undertaken in knwon areas of high incidence, we feel that the resulting information contributes to the need for general knowledge of rabies prevalence in Indonesia. Such findings do not exclude the possibility of partial or total involvement in similar or same environment, nor do they alter the possibility of exposure by individuals in a similar course of events in other geographical surroundings or perhaps in altered circumstances. Only by continued monitoring and survey over prolonged periods of time can the role of wild mammals in rabies be established.

SUMMARY

Rabies virus was not found in 328 wild mammals from 28 locales in Indonesia using the fluorescent antibody technique (FAT) and mouse inoculation. The mammals surveyed and their habitats were described in detail and represented a sampling of the variety of both which occurs throughout the Indonesian Archipelago.

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