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Pathogenic Leptospiras Isolated from Malaysian Surface Waters

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Pathogenic leptospiras (1,424) isolated from natural waters and wet soils in Malaysia comprised 29 different serovars (synonym serotypes). All except two of the serovars had been found previously in Malaysia. The exceptional serovars were *werrasingha*, an Autumnalis serogroup member originally isolated in Ceylon, and a new serovar designated *evansi*. Serovar *evansi* had serological affinities with serovar *ranarum* which was isolated from the kidney of a frog in Iowa. The large variety of serovars found in jungle areas was consistent with similar previous findings of diverse serovar infections in troops who had operated in Malaysian jungles.

McCrumb et al. (8) in a clinical and epidemiological study of 244 cases of leptospirosis in Malaysia directed attention to the jungle environment as "one of the most important natural foci of leptospiral infection." In 1961, Baker (5) initiated studies to determine the infectiousness of waters in the Malaysian rain forest.

For this purpose, Baker and Baker (6) developed a sensitive hamster exposure method for the isolation of pathogenic leptospiras from natural milieu (6). The study was focused principally on the Gombak River and other adjacent streams or rivers located within an area of approximately 20 miles from Kuala Lumpur.

Additional studies were carried out on jungle streams in the State of Pahang and in North Borneo, and on a ricefield and tin mining pool. During the course of these studies, approximately 1,424 pathogenic leptospiras were isolated from waters or wet shore soils. The serological characterization of the isolated strains is described in this report. The epidemiological aspects of the study will be presented separately.

MATERIALS AND METHODS

Origin of strains. Strains were isolated from 1961 to 1966 from five different geographic or ecological sites. Approximately 1,185 strains were isolated from jungle waters and soils within a radius of 20 miles from Kuala Lumpur in the State of Selangor. Fifty-six isolates from ricefields and 17 isolates from pools of water in mining areas were also obtained in the proximity of Kuala Lumpur. The 104 leptospiral strains from the State of Pahang were derived mostly from shore sand along a jungle stream and river located near Kuala Lipis. Jungle streams in the western part of Sabah were the source of 62 leptospiral isolates. Nearly all of the isolates were recovered from hamsters dying from leptospirosis after their exposure to sample water or sample soil washings. The survey method is described elsewhere (6).

Culture typing. The microscopic agglutination test was the basic means for identification of strains. The procedures for this test and also agglutinin-adsorption tests are described in detail elsewhere (2). Generally, strains employed as live antigens were tested for cross-agglutination reactions against the following eight screening serovar (synonym serotype) antisera: *autumnalis*, *alexi*, *medanensis*, *javanica*, *bataviae*, *patane*, *mankarso*, and pooled *australis*, *grippotyphosa*, and *djasiman*. On the basis of initial reactions, strains were subsequently tested against one or more serogroups to determine major antigenic affinities. These tests served to identify strains by serogroup. Representative strains of different patterns of cross-agglutination were then selected for comparative serological tests with type strains of serovars previously found in Malaysia. Isolates were assumed to be serologically homologous with type strains if their cross-reaction patterns were similar. Strains which differed from selected type strains in agglutinogenic characteristics were definitively identified by the use of appropriate agglutinin-adsorption tests (2). The identities of nine other representative isolates were confirmed incidentally for other purposes. The identification of strains was based to large extent on considerable background information obtained in previous studies of pathogenic leptospiras in Malaysia (1, 2).

RESULTS AND DISCUSSION

The 1,362 strains isolated from soil and water in West Malaysia (Selangor and Pahang) could be divided into 13 serogroups comprising a total

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of 29 serovars (Table 1). The wide variety of pathogenic serovars in jungle milieu was consistent with the broad array of serovar infections found in soldiers on jungle patrols (8). Within the State of Selangor, the ricefield and jungle areas studied had similar relative distributions of serovars. However, of 17 strains isolated in mining pools, 16 appeared to be related to serovar *australis* and one to serovar *paidjan*.

The relative frequency of recovered serovar strains in the Pahang jungle superficially differed from those in the Selangor jungle. The variable findings may have reflected differences in size and types of samples and time of sampling. In the limited survey in Sabah mainly *bataviae* and *autumnalis* and a few *icterohaemorrhagiae* serovars were found.

All except four of the representative strains were related to Malaysian serovars previously studied in this laboratory (1, 2). Three of the exceptional strains were identified to be serovars *sentot*, *weerasingha*, and *tarassovi*, respectively, on the basis of agglutinin-adsorption tests. Serovar *sentot* and a member of the Tarassovi serogroup have been reported from Malaysia previously (9). Serovar *weerasingha* was isolated in Ceylon and identified by Y.

TABLE 1. *Pathogenic leptospiras isolated from Malaysian waters and wet soils*

Serogroup	Serovar	Isolates within locations (%)				
		Selangor jungle	Selangor ricefield	Selangor mining pool	Pahang jungle	Sabah jungle
Icterohaemorrhagiae	*mankarso*[a]	18.3	23.2		2.9	8.1
	smithii	2.0				
	birkin	1.1			1.0	
Australis	*australis*[a]	13.5	7.1	94.1	9.6	
Bataviae	*paidjan*[a]	11.6	7.1	5.9	1.9	58.1
	bataviae[a]	3.7				9.7
Canicola	*jonsis*	8.1			1.0	
	schuffneri	4.6	1.8		31.7	
	malaya[a]	0.5				
	sumneri	0.8	3.8		8.7	
Autumnalis	*bangkinang*	4.1	14.3		3.8	17.7
	rachmat[a]	4.1	1.8		7.7	
	gurungi	0.6				3.2
	weerasingha[a]	1.0	1.8			1.6
	sentot[a]	0.6	1.8		3.8	1.6
Pyrogenes	*zanoni*	7.3	8.9		5.8	
	biggis	2.0	1.8		11.5	
	hamptoni	1.5				
	abramis	0.2				
Hebdomadis	*wolffi*[a]	7.2	23.2		5.8	
	ricardi	1.5			1.0	
	worsfoldi	0.1				
Grippotyphosa	*grippotyphosa*[a]	4.9	1.8		3.8	
Javanica	*javanica*[a]		1.8			
Celledoni	*whitcombi*	0.2				
Pomona	*pomona*	0.3				
Tarassovi	*tarassovi*[a]	0.1				
Ranarum	*evansi*[a]	0.1				

[a] The identity of representative strain was confirmed by agglutinin-adsorption tests.

Chernukha (C. Sulzer, personal communication). It had not heretofore been found in Malaysia. It was found in waters of both East and West Malaysia.

The fourth strain (267-1348) cross-reacted to some but not all of the serovars in the Canicola, Icterohaemorrhagia, Javanica, and Autumnalis serogroups, and to homologous titer with a strain isolated from the kidneys of a frog in Iowa (7), and which Babudieri (4) classified as a new pathogenic serovar *ranarum* in a separate serogroup Ranarum (3). In comparative cross-agglutination tests with diverse serovar antisera, the reactions of *ranarum* and strain 267-1348 were essentially identical (Table 2). Reciprocal agglutinin-adsorption tests were carried out between strain 267-1348 and *ranarum*

TABLE 2. *Cross-agglutination reactions of strains 267-1348 and ICF (serovar ranarum)*

Antisera[a]		Reciprocal of titer[b] with:	
Serogroup	Serovar	267-1348	ICF
Icterohaemorrhagiae	*birkini*	400	—
	smithii	400	100
Canicola	*jonsis*	200	100
	broomi	800	200
	bindjei	400	100
	schuffneri	400	200
	benjamin	1,600	800
	malaya	800	400
	galtoni	3,200	800
Pyrogenes	*robinsoni*	1,600	800
Javanica	*javanica*	400	—
	poi	1,600	6,400
	sorex	100	1,600
	ceylonica	—	400
Autumnalis	*djasiman*	1,600	1,600
	gurungi	1,600	1,600
Ranarum	*ranarum* (ICF)	3,200	3,200
	evansi (267-1348)	25,600	3,200

[a] Homologous titers ranged from 1:6,400 to 1:102,400.

[b] Titers of 1:100 or less (—) with the following serovar antisera: *sarmin, icterohaemorrhagiae, copenhageni, mankarso, naam, canicola, kabura, kashirski, portland-vere, zanoni, abramis, pyrogenes, manilae, hamptoni, biggis, sofia, anhoa, celledoni, panama, shermani, medanensis, bataviae, tarassovi, pomona, autumnalis, rachmati, fort-bragg, bangkinang, erinacei-auriti, mooris, sentot, lanka, australis, grippotyphosa, cynopteri, canalzonae.*

TABLE 3. *Cross-agglutinin adsorption studies on strain 267-1348*

Antiserum		Reciprocal of titer[a] with:	
Against	Adsorbed with	Homologous strain after adsorption	Adsorbing strain before adsorption
267-1348 (102400)[b]	*ranarum*	6,400	12,800
267-1348 (25600)	*jonsis*	25,600	1,600
267-1348 (25600)	*broomi*	25,600	1,600
267-1348 (25600)	*benjamin*	25,600	1,600
267-1348 (25600)	*malaya*	25,600	6,400
267-1348 (25600)	*robinsoni*	25,600	1,600
267-1348 (25600)	*javanica*	25,600	1,600
267-1348 (25600)	*poi*	25,600	1,600
267-1348 (25600)	*coxus*	25,600	6,400
267-1348 (25600)	*djasiman*	25,600	1,600
267-1348 (25600)	*gurungi*	25,600	6,400
ranarum (51200)	267-1348	12,800	51,200
jonsis (25600)	267-1348	25,600	400
broomi (102400)	267-1348	102,400	400
benjamin (25600)	267-1348	6,400	1,600
malaya (102400)	267-1348	102,400	1,600
robinsoni (25600)	267-1348	25,600	1,600
javanica (25600)	267-1348	25,600	400
poi (102400)	267-1348	102,400	1,600
coxus (102400)	267-1348	102,400	1,600
djasiman (25600)	267-1348	25,600	6,400
gurungi (25600)	267-1348	25,600	1,600

[a] Cross-agglutinins against heterologous (adsorbing strain) antigens were undetectable at 1:100 dilution after adsorption with respective heterologous antigens. Homologous titers after adsorption with homologous antigen were 1:100 or less except for *javanica, malaya,* and *poi* which were 1:400.

[b] Reciprocals of homologous titers of unadsorbed sera shown in parenthesis.

as well as with various serovar representative strains with which it cross-reacted to relatively high titer. The results of tests are summarized in Table 3. The antibodies responsible for specificity of the test serovars were not removed after adsorption with strain 267-1348 nor was the anti-267-1348 sera significantly reduced after adsorption by any of the test antisera. Accordingly, it represents a new serovar which we designate *evansi.*

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