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## A NEW LEPTOSPIRAL SEROTYPE BELONGING TO SEROGROUP ICTEROHAEMORRHAGIAE\*

ALFRED URQUHART, LOUIS GRANT and CATHERINE SULZER

**Abstract:** The name *monymusk*, strain LT 75-68, has been proposed for a new serotype of *Leptospira* isolated from the kidney tissue of a rat.

### INTRODUCTION

*Leptospira icterohaemorrhagiae* is one of the most common causative organisms of leptospirosis in Jamaica, West Indies. Isolations have been made from humans, rats (*Rattus rattus*, *Rattus norvegicus*), mongooses (*Herpestes auropunctatus*), and house mice (*Mus musculus*).

Serotype *kremastos* of the Hebdomadis group, recently found to be serotype *jules*, was the first serotype to be isolated in Jamaica from man.<sup>1</sup> Since that time, serotypes *icterohaemorrhagiae*, *abramis*, *pomona* and *canicola* have been isolated in Jamaica and identified by the Leptospira Reference (LR) Laboratory at the London School of Hygiene and Tropical Medicine, London, England, and by the Center for Disease Control (CDC), Atlanta, Georgia.

During investigations into possible reservoirs of leptospires among wild animals conducted by the Department of Microbiology of the University of the West Indies, a new strain was isolated from the kidney of a young female rat (*Rattus rattus*). Isolates from other rats (*Rattus norvegicus*) were identified as being identical to the new serotype. This report describes this new strain and its isolation.

### MATERIALS AND METHODS

Live rats trapped in various areas were brought into the University of the West

Indies laboratory. A blood sample was collected by cardiac puncture after the animal had been anesthetized with chloroform and the thoracic cavity opened. The abdominal cavity of each rat was then opened, and the surface of the left kidney was cauterized with a hot scalpel. A sterile Pasteur pipette was then inserted into the kidney to withdraw a small plug of the cortex for aseptic inoculation into one tube each of Fletcher's and Ellinghausen's media.<sup>2,3</sup> This same pipette was then used to inoculate a second tube of the same medium from the same left kidney. The right kidney was then exposed and cauterized, and tissue was withdrawn with another sterile pipette and inoculated into a tube of the medium.

The cultures, incubated at 29C, were first examined by darkfield microscopy on the 5th day for the presence of leptospires. Blood, after standing at room temperature for 3 hours, was centrifuged, and the serum was removed and stored at -20C until needed.<sup>4</sup>

### RESULTS

A culture from kidney tissue of a female rat (*Rattus rattus*) was examined by darkfield microscopy and found to be positive for leptospiral organisms on the 7th day. Antigen was then prepared from culture LT 75-68 (LR 202-68) and tested by cross agglutination with antisera of 13 serotypes (Table 1). Results

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TABLE 1. Cross-agglutination reactions of leptospiral strain LT 75-68 showing relationship with members of the representative serogroups below.

Serogroup	Antiserum		Titer against antigen	
	Serotype	Strain	Homologous	LT 75-68
ballum	ballum	Mus 127	6,400	—
canicola	canicola	Ruebush	51,200	1,600
icterohaemorrhagiae	icterohaemorrhagiae	RGA	25,600	6,400
bataviae	bataviae	Van Tienen	25,600	400
grippotyphosa	grippotyphosa	Adaman	25,600	—
pyrogenes	pyrogenes	Salinem	25,600	400
autumnalis	autumnalis	Akiyami A	25,600	—
autumnalis	autumnalis	Fort Bragg	25,600	—
pomona	pomona	Pomona	25,600	—
australis	australis	Ballico	6,400	—
tarassovi	tarassovi	Perepelicin	—	—
hebdomadis	wolfii	3705	25,600	—
hebdomadis	georgia	LT 117	25,600	—

— = non-reactive

TABLE 2. Cross agglutination reactions of leptospiral strain LT 75-68 with members of the Icterohaemorrhagiae and the Canicola serogroups.

Antiserum		Titer against antigen		LT 75-68 serum
Serotype	Strain	Homologous	LT 75-68	against antigens in Column 1
<i>Icterohaemorrhagiae serogroup</i>				
icterohaemorrhagiae	RGA	25,600	25,600	12,800
mankarso	Mankarso	12,800	6,400	1,600
naam	Naam	12,800	1,600	6,400
sarmin	Sarmin	12,800	1,600	6,400
birkini	Birkin	12,800	400	25,600
smithi	Smith	6,400	400	6,400
ndambari	Ndambari	12,800	1,600	6,400
ndahambukuje	Ndahahbukuje	12,800	6,400	12,800
mwogolo	Mwogolo	12,800	6,400	6,400
dakota	Grant River	12,800	6,400	12,800
weaveri	CZ 390 U	25,600	1,600	1,600
gem	LT 16-67	25,600	1,600	1,600
hualien	LT 1131	25,600	3,200	1,600
<i>Canicola serogroup</i>				
canicola	Hond Utrecht IV	6,400	400	1,600
galtoni	LT 1014	12,800	400	400
bafani	Bafani	12,800	400	6,400
kamituga	Kamituga	25,600	—	1,600
jonsis	Jones	6,400	—	—
sumneri	Sumner	3,200	—	1,600
broomi	Patane	12,800	100	—
bindjei	Bindjei	6,400	—	400
schueffneri	Vleermuis 90C	1,600	—	400
benjamin	Benjamin	12,800	400	400
malaya	H 6	3,200	100	1,600

TABLE 3. Results of cross agglutinin-absorption tests on leptospiral strain LT 75-68 with members of the Icterohaemorrhagiae and Canicola serogroups.

Icterohaemorrhagiae serogroup Serotype	Antiserum	Titer against antigen			
		Homologous		Absorbing strain	
		Before	After	Before	After
icterohaemorrhagiae RGA	LT 75-68	12,800	6,400	12,800	100
mankarso, Mankarso	LT 75-68	25,600	12,800	1,600	100
naam, Naam	LT 75-68	12,800	3,200	800	—
sarmin, Sarmin	LT 75-68	12,800	6,400	6,400	—
ndambari, Ndambari	LT 75-68	51,200	12,800	1,600	—
ndahambukuje, Ndahambukuje	LT 75-68	12,800	12,800	1,600	200
mwogolo, Mwogolo	LT 75-68	12,800	12,800	6,400	100
dakota, Grand River	LT 75-68	12,800	25,600	12,800	—
weaveri, CZ 390 U	LT 75-68	25,600	25,600	3,200	—
LT 16-67	LT 75-68	25,600	51,200	800	—
LT 1131	LT 75-68	25,600	25,600	12,800	200
LT 75-68	icterohaemorrhagiae, RGA	12,800	1,600	12,800	100
LT 75-68	mankarso, Mankarso	25,600	3,200	1,600	—
LT 75-68	naam, Naam	25,600	25,600	6,400	—
LT 75-68	sarmin, Sarmin	25,600	12,800	12,800	—
LT 75-68	birkini, Birkin	25,600	25,600	25,600	—
LT 75-68	smithi, Smith	25,600	12,800	6,400	—
LT 75-68	ndambari, Ndambari	25,600	25,600	6,400	—
LT 75-68	ndahambukuje, Ndahambukuje	25,600	25,600	25,600	—
LT 75-68	mwogolo, Mwogolo	25,600	25,600	6,400	—
LT 75-68	dakota, Grand River	25,600	12,800	12,800	200
LT 75-68	weaveri, CZ 390 U	25,600	6,400	1,600	—
LT 75-68	LT 16-67	12,800	3,200	1,600	—
LT 75-68	LT 1131	25,600	25,600	3,200	100
Canicola Serogroup					
LT 75-68	canicola, HondUtrecht IV	51,200	51,200	400	—
LT 75-68	bafani, Bafani	51,200	25,600	25,600	200
LT 75-68	kamituga, Kamituga	51,200	51,200	1,600	—
LT 75-68	summeri, Summer	51,200	25,600	1,600	—
LT 75-68	malaya, H 6	51,200	25,600	12,800	—

of these tests indicated that this strain was closely related to those of the canicola and icterohaemorrhagiae serogroups (Table-2). Findings in subsequent agglutinin-absorption studies showed that this strain was a new serotype in the icterohaemorrhagiae serogroup (Table 3). The designation, serotype *monymusk*, strain LT 75-68, is proposed.

Two other isolates (LR 192-68 and LR 230-68) obtained by culturing kidney tissues from rats (*Rattus norvegicus*) from Jamaica were confirmed as identical to the new isolate. The serum taken from one of the rats at the time of exsanguination was negative for leptospiral antibodies, but antibodies were present in the other two rats from which the organism was obtained.

#### DISCUSSION

Serotype *monymusk* represents a new isolation from the kidney tissue of a rat in Jamaica, West Indies. Often cultures from the kidney tissue of rats are positive for the leptospiral organism while the sera of these rats are negative for leptospiral antibodies.<sup>6,7</sup> Rats that harbor the organisms in their kidney tubules are potentially dangerous carriers. These organisms may be shed for great lengths of time among animal populations.<sup>5</sup>

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