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Source: Journal of Wildlife Diseases, 13(3) : 286-289

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-13.3.286>

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## LEPTOSPIROSIS IN SMALL MAMMALS OF IRAN: II: ISOLATION OF *Leptospira grippotyphosa* FROM *Mus musculus*

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**Abstract:** The serotype of *Leptospira grippotyphosa*, which is most frequently encountered among sheep, cattle and man in Iran, was isolated from the kidney of a house mouse, *Mus musculus*, by direct culture and animal inoculation. This is the first time that a rodent reservoir of *L. grippotyphosa* in Iran has been investigated and reported.

### INTRODUCTION

In previous investigations on leptospirosis in small mammals of Iran, a *Leptospira* strain belonging to the *L. hebdomadis* serogroup was isolated from the kidney of an *Apodemus sylvaticus* trapped at 65 Km. Northwest of Tehran.<sup>5</sup> The present communication describes the isolation of *Leptospira grippotyphosa* from the kidney of *Mus musculus* captured at Shushtar, 807 km. southwest of Tehran.

### MATERIALS AND METHODS

The small mammals captured in the field were killed, and one kidney from each was removed aseptically. Approximately 0.5 gm. of kidney from each animal was macerated individually by forcing the tissue through the bore of a sterile 2.5 ml plastic syringe and cultured into tubes containing 5 ml of Fletcher's medium. Tubes were labeled and submitted to the Razi Institute to be incubated at 28 to 30 C.

If leptospire became contaminated with other bacteria, a one ml aliquot was inoculated intraperitoneally into each of 2 guinea-pigs. The body temperature of guinea-pigs was recorded

twice a day for 30 days. When guinea-pigs became hyperthermic, one drop of cardiac blood was inoculated into each of 4 tubes of Fletcher's medium. Cultures were then incubated at 28 to 30 C and examined weekly for 6 weeks by dark-field microscopy for leptospire. Cultures were considered negative if leptospire were not found.

The serotype was determined by an agglutination-lysis method. Leptospire were inoculated into two guinea-pigs and at five weeks post-inoculation sera was withdrawn by cardiac puncture. The sera was then tested against 15 serotypes of 7-10 day-old cultures of: *L. australis*, *L. ballum*, *L. autumnalis*, *L. bataviae*, *L. borincana*, *L. butembo*, *L. canicola*, *L. Patoc*, *L. copenhagen*, *L. grippotyphosa*, *L. javanica*, *L. pomona*, *L. pyrogenes*, *L. tarassovi*, *L. wolffii*. Titres of 1:100 or more were regarded as positive.

### RESULTS

A total of 1146 small mammals of 22 species (Table 1), trapped in different field stations in Iran (Figure 1), were examined. In one instance, one *Mus musculus* captured at the Shushtar area

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TABLE 1. Small mammals examined for leptospires in Iran.

Order	Genus and species	Field stations (See map)	Number examined	Number positive	
Rodentia	<i>Apodemus sylvaticus</i>	29, 30, 32, 36	85	—	
	<i>Mus musculus</i>	29, 32, 33, 34, 35, 37, 38, 40, 41, 42, 44, 46	250	1(+)	
	<i>Cricetulus migratorius</i>	29, 32, 35, 38	32	—	
	<i>Mesocricetus auratus</i>	29	5	—	
	<i>Meriones libicus</i>	37, 8R, 38, 39, 41, 42, 44, 46	89	—	
	<i>M. persicus</i>	29, 31, 13R, 33, 34, 19R, 37, 8R, 39, 42, 45, 46	98	—	
	<i>M. crassus</i>	34, 37, 38, 39, 41, 42, 45	68	—	
	<i>Allactaga elater</i>	31, 38	4	—	
	<i>A. williamsi</i>	29, 30, 31	15	—	
	<i>Arvicola terrestris</i>	30, 31, 13R	3	—	
	<i>Calomyscus bailwardis</i>	30, 31, 33, 36, 8R, 39, 43, 46 13R, 8R	78	—	
	<i>Microtus arvalis</i>	8R	5	—	
	<i>M. nivalis</i>	35, 37, 38, 40	5	—	
	<i>Gerbillus nanus</i>	40, 42, 45	164	—	
	<i>G. cheesmani</i>	40, 42, 43, 44	10	—	
	<i>Tatera indica</i>	34, 35, 40, 44	89	—	
	<i>Nesokia indica</i>	34, 35, 40, 44	86	—	
	<i>Jaculus jaculus</i>	35	15	—	
	Insectivora	<i>Crociodura russula</i>	30, 31, 13R	15	—
		<i>Hemiclinus megalotis</i>	40	25	—
Chiroptera	<i>Pipistrellus pipistrellus</i>	38, 39	2	—	
Lagomorpha	<i>Lepus capensis</i>	29, 44	3	—	

(+) *L. grippotyphosa*, from Shushtar (807 Km. Southwest Tehran).

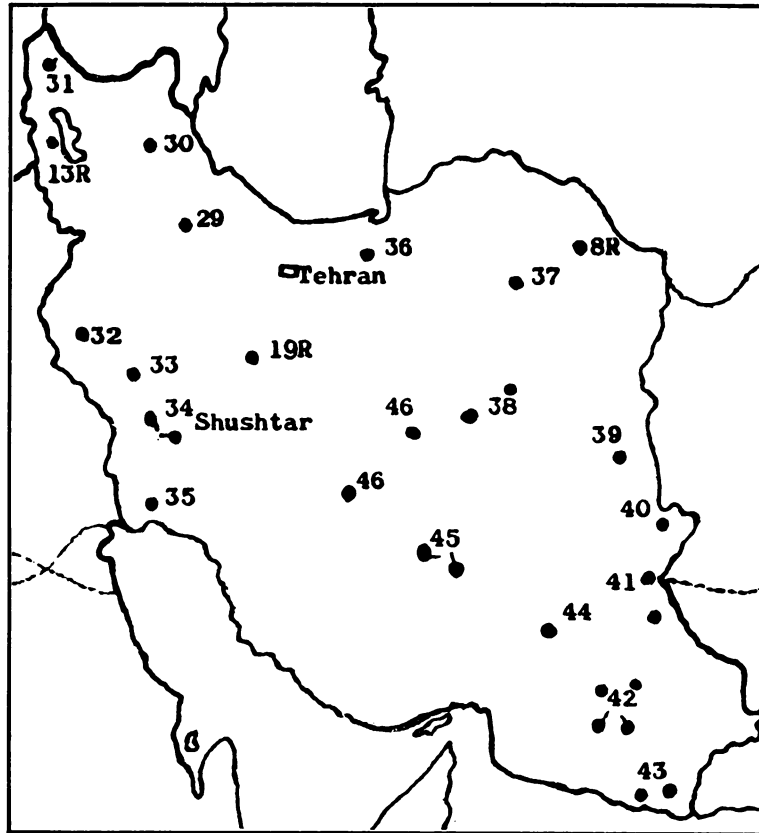


FIGURE 1. Map of Iran

● Field Station (sites of collection of small mammals)

(807 km. Southwest Tehran) a *Leptospira* strain, contaminated with other bacteria, was isolated from the kidney. Two guinea-pigs inoculated with contaminated cultures developed hyperthermia (maximum 40.2 C) from the 5th to 11th day of postinoculation and recovered from the disease. Haemoculture from these guinea-pigs yielded a pure culture of leptospires and a preliminary serologic investigation revealed that the leptospire belonged to the *grippotyphosa* serogroup. The isolate was sent to Dr. A. D. Alexander (WHO/FAO Leptospirosis Reference Laboratory, Walter Reed Army Institute of Research, Washing-

ton, D.C., USA) for typing. The finding was confirmed and the leptospire identified as the *L. grippotyphosa* serogroup.

#### DISCUSSION

Many species of small mammals, especially field rats and mice, are reservoir hosts of prime importance for most serotypes of leptospires in various parts of the world.<sup>2</sup> The serotype of *L. grippotyphosa* has been isolated from *Mus musculus* in USSR,<sup>6</sup> Czechoslovakia,<sup>3</sup> Poland,<sup>7</sup> Israel<sup>4</sup> and Egypt.<sup>1</sup>

In Iran, *L. grippotyphosa* is most frequently encountered among sheep, cattle and man.<sup>8,9,10</sup> Investigations on the reservoir hosts of this serotype showed that *M. musculus*, in Iran, could harbour *L. grippotyphosa* in the kidney and excrete organisms in the urine, thereby transmit-

ting infection to domestic animals and man. It is the first time that the presence of a rodent reservoir of *L. grippotyphosa* in Iran has been investigated. The results indicated a very low prevalence of infection.

#### Acknowledgement

The authors are indebted to Dr. A. D. Alexander for confirming the serotype of isolated leptospire. They also thank the staff of Public Health Institute, Tehran University, for identification of small mammals.

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Received for publication 22 September 1976