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LEPTOSPIROSIS IN SMALL MAMMALS OF IRAN: I. SEROLOGIC TESTS AND ISOLATION OF *Leptospira hebdomadis* FROM *Apodemus sylvaticus*

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Abstract: Leptospires of the *hebdomadis* sero-group and related to *sejroe* serotype, were isolated from the kidney of a vole (*Apodemus sylvaticus*) by direct culture as well as by animal inoculation. Sera of the vole from which leptospires were isolated, and serologic specimens from 1372 other small mammals, were negative for leptospiral agglutinins.

INTRODUCTION

Serologic tests have demonstrated leptospirosis in domestic animals in Iran.[7] This was followed by the isolation of *L. grippotyphosa* from cattle, sheep and man.[8,9] In a joint effort by the World Health Organization (WHO) and the Institute of Public Health, Tehran University, to determine the ecology of small mammals in Iran, the Razi Institute surveyed small animals for leptospirosis. The results of serologic tests, cultures and animal inoculations for the isolation of leptospires, are presented in this paper.

MATERIALS AND METHODS

Isolation of leptospires

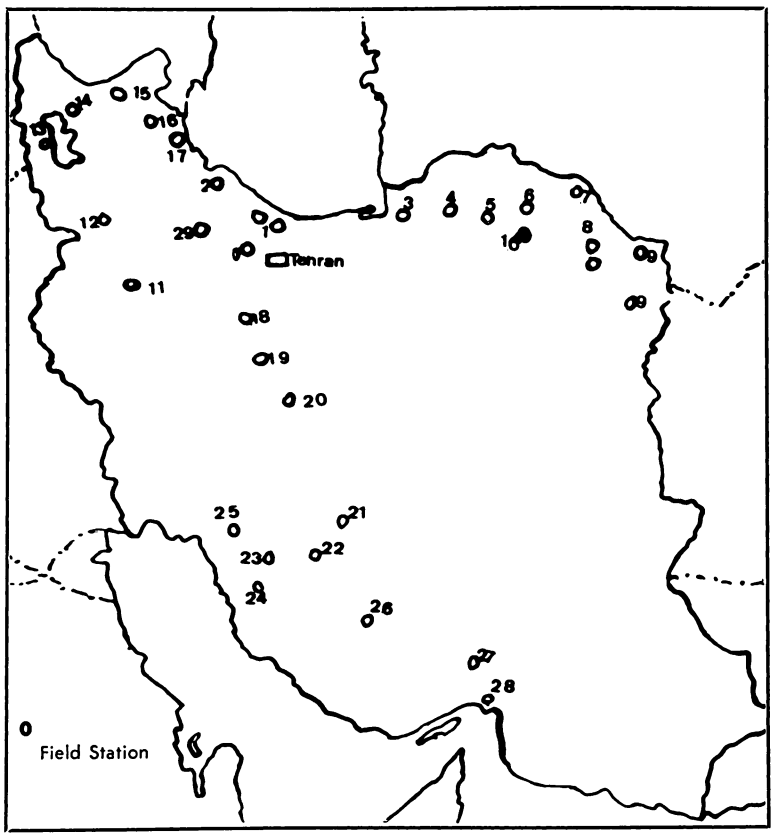
Urine of small animals was examined by dark-field microscopy. They were then killed by chloroform and the kidneys of each animal were removed aseptically and ground by forcing the tissue through the bore of a sterile 2.5 ml syringe into a test tube containing 5 ml physiological saline. Duplicate 0.3 ml aliquots were inoculated into tubes containing 5.0 ml of Kortofs' medium enriched with 10% rabbit serum and incubated at 29 C. In addition, 2 guinea pigs each were inoculated intraperitoneally with 2.0 ml of the kidney suspension. Cultures were examined at weekly intervals for 40 days by dark-field microscopy for leptospires. Cultures were considered negative if leptospires were not found during this period.

Serological tests

Of 1373 serum samples from 45 species (Table 2) evaluated by microscopic agglutination test,[11] 1200 were received as dried whole blood on filter paper. These had been prepared from the heart blood of small mammals trapped in different field stations in Iran (Figure 1). The remaining 173 samples were obtained from blood collected by performing cardiac puncture at the laboratory. To elute serum from dried blood on filter paper, a circular piece of 1.0 cm diameter (approximately 0.02 ml blood) was punched out, cut into small pieces and soaked in physiological saline overnight. After the addition of antigen, which consisted of a fresh rich culture of leptospires, all sera were diluted to 1:50. The microscopic agglutination test was conducted to detect agglutinating antibodies against 10 serotypes of leptospires: *L. autumnalis, L. ballum, L. bataviae, L. canicola, L. pomona, L. grippotyphosa, L. copenhageni, L. pyrogene, L. tarrasovi* and *L.*

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Map of Iran: Sites of collection of small mammals.

wolffi. Sera of 173 live mammals, sent to Razi Institute, were prepared from blood samples obtained by cardiac puncture. Sera was diluted and evaluated in the manner given above.

RESULTS

Table 1 lists the species of small mammals examined for leptospires.In only one case, from *A. sylvaticus,* leptospires were isolated by direct culture of the kidney suspension as well as by haemoculture from the guinea pigs inoculated with the same suspension. Preliminary investigation showed that the isolated leptospire was related to the *hebdomadis* serogroup and this was confirmed by Dr. A. D. Alexander.[2]

Agglutination tests on 1373 serologic specimens of 46 species of small mammals, including the serum sample of the vole from which a leptospire was isolated, were negative.

[2] Chief FAO/WHO Leptospirosis Reference Laboratory, Walter Reed Army Institute of Research, Washington, D.C., USA.

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TABLE 1. Species of Small Mammals Examined for Isolation of Leptospires.

Species	Number examined	Number positive
Dryomys nitedula	2	0
Allactago elater	16	0
Cricetulus migratorius	20	0
Calomyscus bailwardi	4	0
Apodemus sylvaticus	30	1*
Mus musculus	42	0
Microtus socialis	2	0
Meriones persicus	45	0
Meriones lybicus	5	0
Arvicola terrestris	1	0
Ochotona rufescens	4	0
Hemiechinus auritus	2	0

* From 65 km northwest of Tehran.

TABLE 2. List of Small Mammals Examined by Serological Test.

Order	Genus and species	Field stations
Insectivora	*Hemiechinus auritus*	5, 8, 9
	Crocidura suaveolens	3, 21, 25, 28
	C. russula	1, 2, 12, 13, 14, 17
	C. leucodon	4, 19, 20, 21
Chiroptera	*Rhinolophus ferrumequinum*	7, 9
	R. blasii	7
	Tadarida teniotis	7
	Myotis mystacinus	1 ,2, 9
	M. emarginatus	7
	Eptesticus ognevi	7
	E. serotinus	7
	Nyctalus noctula	7, 21
	N. leisleri	2, 7, 9
	Pipistrellus pipistrellus	
	P. savi	7
	Otonycteris hemprichi	7
	Miniopterus schreibersii	7, 8, 9
Lagomorpha	*Lepus capensis*	3, 8
	Ochotona rufescens	6, 7, 9, 10, 19
	Citellus fulvus	8

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TABLE 2 (Continued)

Rodentia	*Allactaga elater*	5, 6, 7
	A. williamsi	11, 15
	Dryomys nitedula	7, 8
	Glis glis	3
	Apodemus sylvaticus	0, 8, 11, 16, 18, 19, 22
	Rattus rattus	2, 17, 28
	Mus musculus	All stations except 7 and 21
	Acomys demidiatus	24, 27, 28
	Nesokia indica	10, 18, 20, 24, 25
	Calomyscus sp.	5, 10, 11, 18, 28
	Cricetulus migratorius	4, 6, 8, 10, 11, 16, 18, 27
	Mesocricetus auratus	11
	Gerbillus nanus	24, 28, 29, 27
	Tatera indica	22, 28
	Meriones persicus	4, 5, 7, 15, 17, 28
	M. teristrami	18, 21, 24
	M. lybicus	5, 8, 9, 18, 19, 26
	M. crassus	5, 10
	Rhombomys opimus	5
	Ellobius fuscocapillus	19
	Arvicola terrestris	13, 20
	Microtus arvalis	4
	M. nivalis	8, 15
	M. socialis	4, 5, 6, 8, 9, 22
	M. transcaspicus	8, 9

DISCUSSION

L. hebdomadis from *A. sylvaticus* has been reported from Italy,[6] Germany,[10] Hungary[2] and Czechoslovakia.[3,4] The leptospire isolated in most cases was related to *L. sejroe*. This is the first report of this sero-group and its reservoir in Iran.

Serum samples collected as dried blood on filter paper had been used previously in serologic tests for detection of leptospiral infections.[12] However, serologic tests on 1200 serum samples obtained as dried blood on filter paper as well as 173 samples of fresh serum prepared at the laboratory, did not reveal leptospirosis. This can be interpreted as a very low prevalence of leptospirosis in small mammals in Iran. The negative result of the microscopic agglutination test on the serum of *A. sylvaticus*, from which a leptospire was isolated, poses the question of whether the serologic tests available to us are reliable for detection of leptospirosis in reservoirs. The phenomenon of culturally-positive, serologically-negative rodents was observed repeatedly by Minette.[5] He explains this phenomenon by recourse to the "ectoparasitism" hypothesis advanced by Babudieri[1] who postulates that an initial stage of leptospiraemia is followed by an immunized state. The organisms begin to accumulate in the distal convoluted tubules of the kidney, and at this point the leptospires cease stimulating the antibody-forming mechanisms of the parasitised animals and, apparently for reasons as yet unknown,

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antibodies previously formed in the blood stream do not affect the organisms. The blood antibody level gradually diminishes until it is no longer detectable by present serological methods. Apparently to determine the actual status of leptospirosis in small mammals and to identify possible reservoirs it is advisable to depend on direct detection of leptospiruria and the isolation of the organism by culture or animal inoculation.

Acknowledgements

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