Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1973, **49**, 597-603

First detection of tularaemia in domestic and wild mammals in Iran *

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During a study on the ecology of small-mammal-borne infections in Iran, over 4 600 wild mammals were collected at 47 localities. Attempts were made to isolate Francisella tularensis *from the spleens of 3 548 of these animals. All were found to be negative. In addition, sera from 200 sheep and cattle and from 39 wild mammals were tested: 8 sheep, 3 oxen, and 1 hedgehog showed evidence of recent infection. This is the first report of tularaemia in Iran. The relationship of these findings to the potential distribution of natural foci in Iran and adjacent countries indicates that the infection in Asia may be more widespread than was previously thought.*

This report records the first observations of tularaemia in Iran and proposes the potential distribution of the infection within the country and neighbouring areas as a basis for future studies.

Tularaemia is widely distributed throughout the Northern Hemisphere, where it is found most often on the plains and sporadically in mountainous regions (8, 20). In Asia, apart from the USSR, the distribution of the agent (*Francisella tularensis*) is little known, as tularaemia has been reported only in eastern Turkey, China, and Japan (22).

The existence of the agent may be determined in man or other mammalian species, arthropods, the aquatic environment, burrow and nest substrata, and the pellets of predatory birds. In areas adjacent to Iran the occurrence of tularaemia has been known for many years. In Turkey the agent has been isolated from human beings (2, 6, 12) and from rodents and stream water (3, 23). In Armenia, infections in man have been related to ticks and mites, rodents, slaughtered sheep, and water (28).

In the Azerbaijan SSR, the infection has been studied since 1958 and many strains of *F. tularensis* have been isolated from wild mammals, ticks, fleas, and water (M. G. Ahundov, unpublished observations, 1969). To our knowledge, the presence of tularaemia has not been reported in other countries adjacent to Iran (Iraq, Afghanistan, and Pakistan). In the Turkmenian SSR, the only natural focus of tularaemia known is in the lower reaches of the Amu-Darya river, several hundred kilometres from the Iranian frontier. Olsulf'ev (19) believed that tularaemia might be more widely distributed and might occur in other parts of southern Central Asia.

Since tularaemia infection occurs in animal populations, its prevalence is very high during epizootic periods. During interepizootic periods, however, its prevalence is so low that the agent of infection is extremely difficult to isolate.[a]

MATERIALS AND METHODS

During 1969 and 1970, we collected 4 600 small wild mammals at 47 sites in Iran (Fig. 1). Spleens were taken in the field, placed individually or in groups of 4–6 in a suitable transport medium (4), cooled, and

* This report is one in a series resulting from an ecological study of certain small-mammal-borne infections, conducted by the Iran–WHO International Epidemiological Research Centre, Teheran, Iran.

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[a] Following tularin-positive skin tests during the summer of 1973 in western Iran, 1 of 13 human sera responded positively to agglutination and haemagglutination tests conducted at the Gamaleya Institute, Moscow. This was the first indication of human tularaemia in Iran (Y. Karimi, personal observation, 1973).

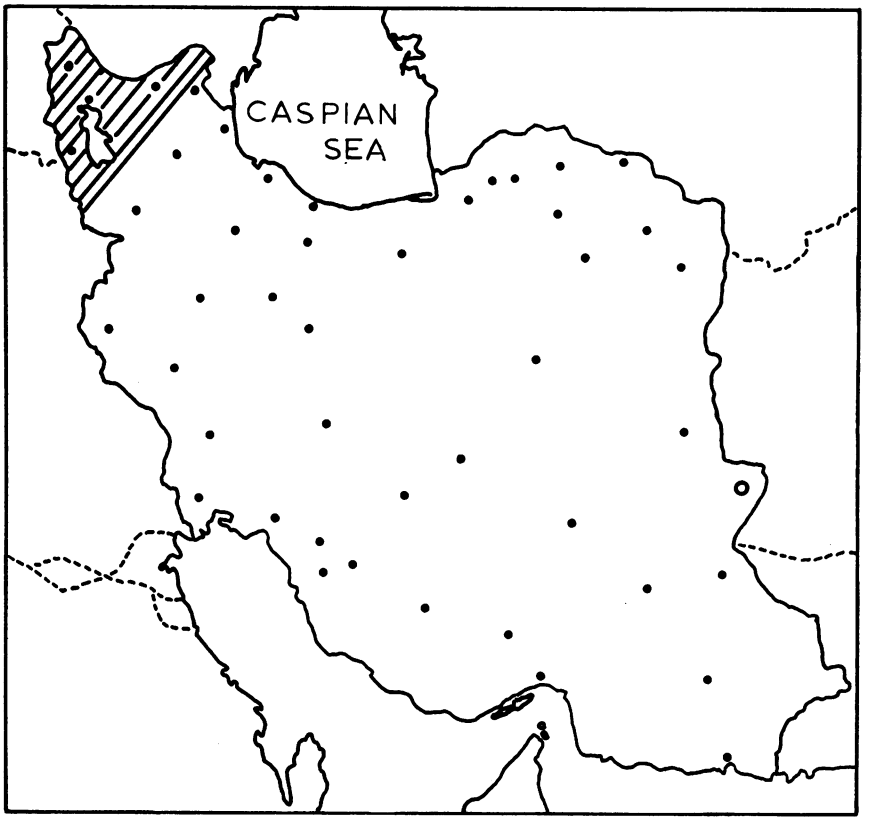

Fig. 1. Map of Iran showing localities from which small mammals were examined for indications of *F. tularensis* (solid circles). Positive serological results were found in a wild hedgehog from eastern Iran (open circle), and in cattle and sheep from Azerbaijan (hatched).

transported at intervals to Teheran. At the Pasteur Institute, Teheran, a suspension made with 3 ml of physiological saline was inoculated subcutaneously into adult guinea-pigs, which were subsequently observed for 20–25 days. In all, 3 548 spleens from 61 species of wild mammal (33 rodents, 21 bats, 5 insectivores, and 2 lagomorphs) were examined. The same material was used simultaneously for the diagnosis of plague [a] and other bacterial infections.

The transport medium was tested in the laboratory and found to be reliable (Table 1). In 1970, another medium (Cary and Blair) was tested in field conditions, with similar results.

In early October 1970, 200 sera from domestic mammals (100 cattle and 100 sheep) were collected at the Teheran slaughterhouse. The animals came from

[a] One strain of plague was isolated from *Meriones persicus*.

Table 1. Results of laboratory tests in which bioassay mice were inoculated with spleen homogenates of mice infected with tularaemia and erysipeloid [b]

Pathogen	Duration of storage (days)	No. of test mice	Storage temperature of media & days on which mice died	
			+ 4°C	+ 18–20°C
F. tularensis	30	4	days 5 & 6 [c]	neither died [d]
E. rhusiopathiae	35	4	day 3 (both) [c]	day 3 (both) [c]

[b] These homogenates were stored at different temperatures in Broquet medium, as modified by Baltazard et al. (4). The tests were conducted in the Tularaemia Laboratory, Gamaleya Institute, Moscow.

[c] The tested pathogen was isolated from dead mice.

[d] The mice did not die as a result of the test and *F. tularensis* was not isolated from them when they were killed.

Table 2. Results of serological testing of 239 domestic and wild mammals for tularaemia

Species	Origin	No.	No. positive	Titres					
				Agglutination			Passive haemagglutination		
				1/10	1/20	1/40	1/80	1/160	1/320
Domestic cattle	north-western Iran	100	3	2	1	—	—	3	—
Domestic sheep	north-western Iran	100	8	4	2	2	1	5	2
Pica *(Ochotona rufuscens)*	Delijan area (central Iran)	22	0	not checked			—	—	—
Gerbil *(M. persicus)*	Delijan area (central Iran)	3	0	»			—	—	—
Gerbil *(M. persicus)*	Mashad area (north-eastern Iran)	3	0	»			—	—	—
Vole *(M. arvalis)*	Mashad area (north-eastern Iran)	1	0	»			—	—	—
Hedgehog *(H. megalotis)*	Zabol area (extreme eastern Iran)	10	1	»			—	1	—

north-western Iran, but details of their ages, history, and means of transport to Teheran are not known. These sera, as well as sera from 39 small wild mammals that had remained after the completion of tests for arboviral and rickettsial infections, were sent to the Tularaemia Laboratory, Gamaleya Institute, Moscow, for serological testing. The species from which sera were obtained are given in Table 2, together with the regions from which they originated.

Agglutination and passive haemagglutination tests for the detection of tularaemia were conducted according to the procedures outlined by Meščerjakova & Ivanova (15).

The agglutination tests were carried out by the standard volume technique (0.5 ml) with an initial serum dilution of 1 : 10. The antigen was prepared from the strain Schu in the Tularaemia Laboratory of the Gamaleya Institute. To control the specificity, an agglutination test with the brucellosis antigen was performed simultaneously.

The passive haemagglutination test was performed with sera diluted as mentioned above, inactivated by warming for 30 min at 56°C, and exhausted by native sheep erythrocytes. The antigen was formalinized sheep erythrocytes sensitized with tularaemia antigen. To each serum dilution 2.5 ml of working suspension of sensitized erythrocytes were added. The results were observed after 2–3 h. The specificity of positive reactions was controlled by the use of the passive haemagglutination inhibition test. To dilutions of each positive serum (0.25 ml) the same amount of tularaemia antigen was added, allowed to stand for 1 h at room temperature, and exposed to sensitized erythrocytes. The results were noted after 2–3 h. A decrease in haemagglutinin activity following the addition of the tularaemia antigen confirmed the specificity of the passive haemagglutination test.

RESULTS

Our attempts to isolate tularaemia direct from the 3 548 spleens of small wild animals were unsuccessful.

However, of the 200 domestic-animal sera examined, 11 were considered to be positive (Table 2) according to the following criteria: (1) all were positive to tularaemia in both agglutination (1 : 10–1 : 40) and passive haemagglutination (1 : 80–1 : 320) tests; (2) the agglutination test with the brucellosis antigen was negative in all cases; and (3) specificity was demonstrated by the haemagglutination inhibition test.

Of the 39 wild-mammal sera examined, one was positive (1 : 160) by the passive haemagglutination test (Table 2). These sera were not checked by the agglutination test as were those from domestic animals. However, they were also examined for plague and were found to be negative by the passive haemagglutination test with plague erythrocyte antigen produced by the Alma-Ata Central-Asian Anti-Plague Institute.

DISCUSSION

The landscapes of Iran are varied. Much of the country is covered by mountain ranges with an altitude of over 900 metres. The Caspian region has a humid climate and deciduous woods, but there is little vegetation on the vast plains of the central Iranian deserts (Dasht-e-Kevir and Dasht-e-Lut). There are sparse forests of oaks and pistachios on the mountains of Iranian Azerbaijan and in the Zagros mountains. Near the coast of the Persian and Oman gulfs a tropical influence is indicated by the presence of mangrove marshes. The rest of Iran is a mixture of semidesert and mountain-steppe regions (29).[a] The mammalian fauna is also diverse, with a nucleus of endemic species augmented by numerous species that have penetrated from the North, West, and East (9, 14, 16, 17). There is a broad drainage pattern, but owing to the scarcity of rainfall the rivers are shallow except for those flowing down from the Elburz range to the Caspian Sea and from the Zagros range into the Persian Gulf.

So far, natural infection with the agent of tularaemia has been confirmed in 125 vertebrate and 101 invertebrate species (22). Observations in natural foci and special laboratory experiments show that, according to the degree of susceptibility and infective sensitivity to tularaemia, three groups of mammals can be distinguished (21, 22). Group I includes species with high susceptibility and high sensitivity; group II, species with high susceptibility but low sensitivity; and group III, species with low susceptibility and practically no sensitivity. Animals of group I are infected by subcutaneous inoculation of individual cells of the tularaemia microbe. The disease runs an acute course and the intensive multiplication of microbes in parenchymatous organs enhances the regular transmission of the agent from sick to healthy individuals (7). The transmission of infection by animals of group II is lower, and by animals of group III (mainly belonging to the orders of the *Carnivora* and *Ungulata*, including domestic cats and dogs and farm animals), it is almost impossible.[b] Therefore to analyse the circumstances in which natural foci of tularaemia occur in Iran, we must consider the distribution of mammals belonging to group I.

In the USSR, 50 species (including 43 rodents, 3 hares, and 4 insectivores) fall into this category (22). According to Lay (14) the following species of group I are found in Iran: *Arvicola terrestris*, *Microtus arvalis* and *M. socialis*, *Mus musculus*, *Apodemus sylvaticus*, *Cricetulus migratorius*, *Mesocricetus auratus*, *Meriones libycus* and *M. meridianus*, *Rhombomys opimus*, and various hares whose nomenclature is poorly known. Other species not occurring in the USSR will obviously be found if further studies are conducted in other Asian countries. Furthermore, the taxonomy of many of the groups (e.g., hares and microtines) is not satisfactorily agreed upon, as can be seen from the literature (5, 14, 16).

Fig. 2 shows the published distribution of group I species occurring in Iran, in relation to tularaemia, according to Misonne (16). The distribution of *M. musculus*—which is almost universal in Iran—is not shown. Rodents such as *Mus* and *C. migratorius* often inhabit human dwellings and may regularly migrate to and from neighbouring natural habitats (13, 25, 27), potentially transporting infections from natural foci to human settlements.

The ixodidae ticks—the main vectors and spontaneous carriers of tularaemia—are common in Iran, more than 20 species having been reported so far (1, 10). Spontaneous infection with tularaemia has been reported outside Iran in *Dermacentor marginatus*, *Haemaphysalis concinna*, *H. punctata*, and *H. sulcata*, *Hyalomma asiatica*, and *Ixodes ricinus*. It is reasonable to assume that these species could also maintain the preservation and circulation of tularaemia in the natural conditions of Iran. In addition to these species, there is in Iran a wide variety of blood-sucking insects that may participate in the transmission of tularaemia. As has already been observed in Armenia (24, 26), the infected ixodidae ticks that infest sheep may be one of the sources of infection of human beings. The danger of infection from that source increases as flocks of tick-infected sheep are driven over great distances, the ticks thus being carried away from their natural foci to other regions. Similar observations have also been made in the USA (11).

In view of the natural occurrence of known wild reservoir and vector species in Iran; the presence of large numbers of sheep, goats, and cattle; and the reports of tularaemia infection from adjacent and ecologically similar areas, it seemed reasonable to expect to find the infection in Iran during the present study.

We failed to isolate tularaemia from the spleens of

[a] This is apparent also from the map of the natural vegetation of Iran, developed by S. Mobayen and V. Tregubov and published by the University of Teheran in 1970.

[b] Human response to tularaemia, according to T. N. Dunaeva (unpublished observations, 1968), corresponds most closely to that of group II animals.

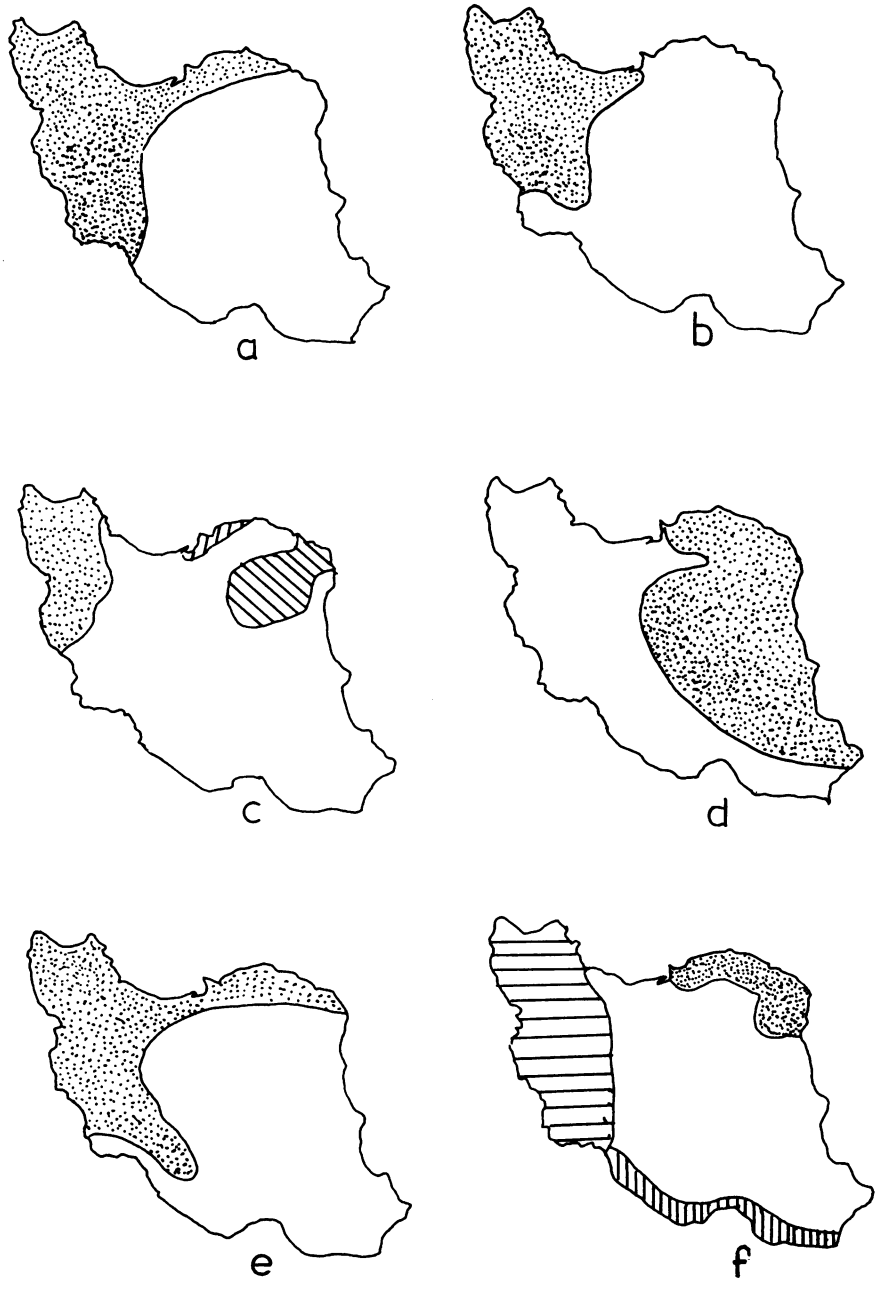

Fig. 2. Distribution of some mammals in Iran belonging to the group of animals with high susceptibility and high sensitivity to tularaemia, according to Misonne (16) and modified by reports from Lay (14) and the results of the present study: (*a*) Genus *Microtus* (4 species: *M. arvalis, M. transcaspicus,* and *M. socialis* in the north, and *M. irani* in the west); (*b*) *A. terrestris*; (*c*) *M. auratus* (stippled) and *M. meridianus* (hatched); (*d*) *R. opimus*; (*e*) *A. sylvaticus*; and (*f*) Genus *Lepus*: *L. europaeus* (horizontal hatching), *L. arabicus* (vertical hatching), and *L. capensis* (stippled).

over 3 500 wild mammals, although these included large numbers of *M. persicus* (613), *M. musculus* (591), and *A. sylvaticus* (304). Furthermore, the transport medium was laboratory-tested and found to be satisfactory. Subsequent serological tests suggested that our sample of wild mammals was probably too small, since for successful isolation of tularaemia, even in known foci, large samples captured over a long period are needed.

The relatively large number of positive results that we obtained from cattle and sheep material (Table 2) demonstrates that tularaemia occurs in Iran. In view of the prevailing conditions for its natural occurrence it seems likely that tularaemia also occurs in man. Until further studies have been conducted, the extent to which the disease poses a public health problem will remain unknown.

The 39 wild-mammal sera tested came from a number of localities (Table 2). Although the sample is small, the single positive result from eastern Iran (near Zabol, in the valley of the Hirmand River) is interesting. Ten hedgehogs (*Hemiechinus megalotis*) were sampled here in early November 1970. The serum of one of them was positive by haemagglutination and the specificity was confirmed by the haemagglutination inhibition test, suggesting that the tularaemia infection had been contracted in the early autumn. The hedgehogs at this locality were heavily infested with ticks of an undetermined species. This record is of interest primarily because it concerns the southernmost occurrence of the infection observed in Asia and, if confirmed, it could change many ideas about the limits of tularaemia in the Palearctic. Thus it appears that there is a latent focus of tularaemia in the Seistan depression that is completely isolated from other known foci in the Middle East. Our mammal collections at this site included *M. musculus*, *C. migratorius*, *Nesokia indica*, and hares, from which strains of tularaemia have been isolated in the tugai focus in Central Asia—a type of focus characteristic of desert rivers (18). This focus is several hundred kilometres to the north-east of Iran in the lower reaches of the Amu-Darya and Sir-Darya, and in the Chu Valley. In addition, we collected near Zabol fairly large numbers of such " southern " species as *Tatera indica*, *Gerbillus nanus*, and *G. cheesmani*. As the greater part of the Seistan depression is situated in southern Afghanistan and Pakistan, it is possible that tularaemia may exist in these countries along the valleys of the Hirmand River and other rivers ending in the Hamuni-Hirmand Lakes.

Finally, if tularaemia indeed exists in such sharply different regions as north-western and eastern Iran, as our serological data would indicate, it probably also occurs in central Iran. The most likely place where there might be a focus is the valley of the Zajanderud River, where we observed in the autumn of 1969, 30 km to the south of Isfahan, dense populations of the water vole (*Arvicola terrestris*)—one of the principal carriers of tularaemia in Eurasia, where it coexists with *N. indica*, *M. persicus*, *M. musculus*, and other susceptible rodent species.

ACKNOWLEDGEMENTS

The authors thank all those who assisted them in this study, especially Mr P. Goudarzian, Mr M. A. Afshar, Mr H. Mahmoodi, Mr M. Sabetzadeh, Mr N. Malakuti, Mr A. Mahalati, and Mr H. Novrian, who worked in difficult field conditions.

They also gratefully acknowledge the continuing support of Professor C. Mofidi, Co-director of the Iran–WHO International Epidemiological Research Centre, and the assistance received from Dr M. Faghih, Director, Institute of Public Health Research, University of Teheran; Professor V. Kučeruk, Chief, Department of Natural Focality of Diseases, Gamaleya Institute, Moscow; and Professor N. G. Olsuf'ev and Dr T. N. Dunaeva, of the Tularaemia Laboratory, Gamaleya Institute.

RÉSUMÉ

MISE EN ÉVIDENCE, POUR LA PREMIÈRE FOIS, DE LA TULARÉMIE CHEZ DES MAMMIFÈRES DOMESTIQUES ET SAUVAGES EN IRAN

La tularémie est largement répandue en Turquie, en Arménie et dans la République socialiste soviétique d'Azerbaïdjan, notamment dans les districts voisins de l'Iran. Dans la République socialiste soviétique du Turkmenistan, son foyer naturel est situé dans le bassin de l'Amu-Darya. Des données zoologiques et géographiques

donnent à penser que la maladie existe aussi en Iran, en particulier dans la partie nord-ouest du pays.

En 1969-70, on a capturé 4600 petits mammifères sauvages en 47 endroits de l'Iran et on a tenté d'isoler par inoculation au cobaye, à partir de la rate de 3548 d'entre eux, l'agent responsable de la tularémie, *Francisella tularensis*. Cette recherche n'a donné aucun résultat. On a d'autre part examiné par les épreuves d'agglutination et d'hémagglutination passive les sérums prélevés chez 100 bovins et 100 moutons. Trois sérums de bovins et 8 sérums de moutons ont donné une réaction positive pour l'antigène *F. tularensis*. Enfin, sur 39 sérums de mammifères sauvages, un, prélevé chez un hérisson *(Hemiechinus megalotis)*, renfermait des anticorps antitularémiques.

De nouvelles recherches sur les caractéristiques géographiques des foyers naturels de tularémie en Iran et dans les pays voisins seraient utiles. Il faudrait aussi entreprendre une enquête épidémiologique pour déceler l'existence éventuelle de l'infection chez l'homme et procéder à des collectes d'Ixodidés en vue de l'isolement et de l'étude des souches de *F. tularensis*.

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