

Rickettsioses studies

1. Natural foci of rickettsioses in the Armenian Soviet Socialist Republic

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Ixodid ticks collected in the Armenian SSR during 1971 and 1974 were positive for rickettsiae of the spotted fever group, as confirmed by haemocyte tests and by isolation experiments. Serum specimens collected from human beings and from domestic and wild animals in the same areas contained antibodies against such rickettsiae and against Coxiella burnetii. These results indicate the existence of mixed natural foci of rickettsioses of the spotted fever group and of Q fever in the Armenian SSR.

Investigation of the natural foci of endemic rickettsioses in the Armenian SSR started in 1950. Several human cases of Q fever (8) and of spotted-fever-like infection (6) were found and rickettsial strains similar to those of the spotted fever group were isolated from *Dermacentor marginatus* ticks collected on cattle, from *Rhipicephalus sanguineus* ticks collected on sheep and dogs, and from the brains of hamsters (7, 9, 10).

In view of the new observations recorded recently, ideas have changed markedly as regards the geographical distribution of rickettsioses of the spotted fever group in parts of Europe and Asia. Natural foci of tick-borne typhus were found in the Tula region (11) and in Turkmenistan (12). Insufficiently characterized rickettsiae belonging to the spotted fever group were isolated in Turkmenistan (12), in Czechoslovakia (1), and in Pakistan (17). There is a need for proper identification and classification of the rickettsial strains isolated, in particular in eastern Europe, where the areas of occurrence of tick-borne typhus, boutonneuse fever, and rickettsial-pox may overlap. However, the methods previously

used for interspecies differentiation of rickettsiae of the spotted fever group were inadequate to determine the species of rickettsial strains isolated in the Tula region, in Turkmenistan, and in Czechoslovakia.

To contribute to knowledge of the ecology of spotted fever group rickettsiosis and to the characterization of its causative agent, Soviet and Czechoslovak rickettsiologists carried out two field studies in the Armenian SSR in 1971 and 1974. The results of field and laboratory investigations of natural foci of endemic rickettsioses in different regions of that territory are presented in this paper.

COLLECTION OF MATERIALS

A total of 894 ticks was collected in 7 districts of the Armenian SSR at the end of September 1971 and in 3 districts at the beginning of October 1974. A detailed description of the localities in question is given in Table 1. The ticks were collected either by trailing a white woollen blanket over pastures (in 1971) or from sheep (in 1971 and 1974). The ticks were kept in closed test-tubes at 4°C until required for examination.

Small mammals were captured in special traps (4). Blood samples for serological examination were taken from the orbital sinus.

Human sera were obtained from hospitals or from hygiene and epidemiology stations in the Leninakan and Erevan districts. Cattle and sheep sera were

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Table 1. Areas of the Armenian SSR where ticks and small rodents were collected

District	Locality	Habitat	Landscape characteristics	Altitude (m)
Ahurjan	Leninakan	mountainous steppe	cultivated steppes	1500
Artik	Šnaberd	mountainous steppe	wild steppes	1900
Amasija	Karavansaraj	mountainous steppe	wild steppes	1900
	Šurabad	mountainous steppe	meadow steppes	2200
Aparan	Tudžur	alpine	alpine meadows	2500
Ararat	Čimankend ^a	mountainous steppe	meadow steppes	2000
Aštarak	Koš	semidesert	semicultivated desert	700–800
Sevan	Tzovagjuh	mountainous forest	wild steppes	1900
Ečmiadzin	Samagar	desert	semicultivated desert	700–800
Kafan	Arcvanik ^a	mountainous forest	semicultivated desert and rare forests	1500
Sisijan	Borisovka ^a	mountainous steppe	meadow steppes	2500

^a Collections took place in 1974. In all other localities, collections took place in 1971.

supplied by abattoirs in Leninakan and Erevan, to which animals are brought for slaughter from 9 districts (Kalinin, Gukasjan, Ahurjan, Ararat, Masin, Abovjan, Ečmiadzin, Amasija, and Sisijan). All sera were stored at -20°C .

LABORATORY STUDIES

Detection of rickettsiae in ticks, and isolation of rickettsial strains

The ticks were sorted into groups of twenty according to species, stage, and sex. Each group of ticks was allowed to feed on guinea-pigs or rabbits, which were observed for symptoms of rickettsioses for 3 weeks. The excreta of partly fed ticks were tested further for the presence of rickettsiae by inoculation into guinea-pigs. All unfed and partly fed ticks were examined for infestation with rickettsiae by the haemocyte test (14) with the Giménez staining method and by parallel immunofluorescence testing.

After the ticks had been thoroughly washed in 70% ethanol, repeatedly rinsed in sterile distilled water, and ground, suspensions in phosphate-buffered saline (PBS), pH 7.2, without antibiotics, were prepared from each of the unfed ticks that had been found to be negative in the haemocyte test, for intraperitoneal injection into guinea-pigs. Partly fed ticks that had been found to contain rickettsiae in the

haemocyte test were washed in the same way and a suspension from each tick was prepared in 1.5 ml of PBS containing 100 IU of penicillin and 100 μg of streptomycin. Then 0.25 ml of each suspension was injected into the yolk sac of a 6–7-day-old chick embryo. In almost every case the embryo died 4–6 days after infection and rickettsiae were present in yolk sac smears. Blind passages were not performed.

Serological examination

All human and animal sera were examined by complement fixation or by the microagglutination test. The former was performed with corpuscular phase-II *C. burnetii* antigen for the detection of Q fever antibodies and with spotted fever group-specific soluble antigen for the detection of antibodies to rickettsiae of that group. The microagglutination test was performed as described elsewhere (5) with stained corpuscular phase-II *C. burnetii* converted antigen or stained corpuscular antigen of spotted fever group rickettsiae. Antigens were prepared in the Gamaleja Institute of Epidemiology and Microbiology, Moscow, in 1971, and in the Institute of Virology, Slovak Academy of Sciences, Bratislava, in 1974. Human sera and the sera of small rodents were also subjected to the microagglutination test with stained *Rickettsia canada* antigen.

RESULTS

As shown in Table 2, rickettsiae or rickettsia-like organisms were found by Giménez staining in 163 (18.2%) of the 894 ticks collected. However, fewer ticks were found positive by parallel immunofluorescence testing: 23 (2.6%) containing *C. burnetii* and 105 (11.7%) containing spotted fever group rickettsiae. Thirty-seven strains of the spotted fever group were isolated from *D. marginatus* and 2 strains from *D. reticulatus*.

Confirmation that ticks collected in 1971 were infected with rickettsiae of the spotted fever group was obtained when guinea-pigs that had been subjected to tick bites or inoculated with tick excreta developed symptoms of rickettsial disease and anti-

body response (Table 3). Further investigation in guinea-pigs of ticks that had been found to be negative in the haemocyt test revealed rickettsiae of the spotted fever group in 4 out of 12 groups consisting of 10–30 ticks each. In these cases the ticks were of the *D. marginatus* species, collected in Čimankend (district of Ararat).

The results of the serological examinations are given in Table 4. The most important finding was the presence of antibodies to spotted fever group rickettsiae in human sera. A higher prevalence of such rickettsiae and of *C. burnetii*, as confirmed by a greater proportion of positive human and animal sera, was found in 1974. The antibody titres in all sera tested ranged between 4 and 16, except for two sheep sera with titres of 32 to *C. burnetii*. Human

Table 2. Infection with rickettsiae of ticks collected from sheep in the Armenian SSR

District	Locality	Tick species	No. of ticks investigated		No. of ticks containing rickettsiae as detected by:						No. of strains of spotted fever group isolated			
					Giménez staining				immunofluorescence technique					
					females		males		<i>C. burnetii</i>				spotted fever group	
					females	males	females	males	females	males			females	males
Artik	Karavansaraj	<i>D. marginatus</i>	26	7	4	2	1	—	2	2	—	1		
	Šnaberđ	<i>D. marginatus</i>	55	48	5	7	—	3	4	3	6	2		
		<i>H. sulcata</i>	1	—	—	—	—	—	—	—	—	—		
Aparan	Tudžur	<i>D. marginatus</i>	3	7	2	—	1	—	1	—	—	—		
Ašarak	Koš	<i>D. marginatus</i>	3	5	—	3	—	2	—	2	—	—		
Amasija	Surabad	<i>D. marginatus</i>	18	9	3	2	1	2	1	1	—	—		
Ahurjan	Leninakan	<i>D. marginatus</i>	28	18	8	5	—	—	7	4	4	—		
Ararat	Čimankend	<i>D. marginatus</i>	102	40	14	7	2	1	13	6	10	—		
		<i>H. sulcata</i>	36	2	2	—	—	—	1	—	—	—		
Ečmiadzin	Samagar	<i>D. marginatus</i>	78	15	19	3	1	1	15	3	7	1		
		<i>H. sulcata</i>	11	1	—	—	—	—	—	—	—	—		
Kafan	Arcvanik ^a	<i>D. marginatus</i>	13	7	—	1	—	—	—	1	—	1		
		<i>H. sulcata</i>	12	7	—	—	—	—	—	—	—	—		
Sevan	Tzovagjuh	<i>D. reticulatus</i>	208	73	49	19	8	—	24	8	2	—		
	Sevan ^a	<i>D. reticulatus</i>	18	4	—	—	—	—	—	—	—	—		
Sisijan	Borisovka ^a	<i>D. marginatus</i>	28	10	4	4	—	—	4	3	4	1		
		<i>H. sulcata</i>	1	—	—	—	—	—	—	—	—	—		
Total			641	253	110	53	14	9	72	33	33	6		

^a Ticks collected in 1974. In other localities, the collections took place in 1971.

Table 3. Evidence of rickettsiosis and antibody response in guinea-pigs bitten by ticks or inoculated with their excreta

Locality of tick collection (district)	Tick species	Symptoms of rickettsiosis and antibody response after tick bite				Symptoms of rickettsiosis and antibody response after inoculation of tick excreta			
		fever	scrotal reaction	antibodies ^a to:		fever	scrotal reaction	antibodies ^a to:	
				<i>C. burnetii</i>	spotted fever group			<i>C. burnetii</i>	spotted fever group
Tzovagjuh (Sevan)	<i>D. reticulatus</i>	—	—	—	128	—	+	—	64–128
Leninakan (Ahurjan)	<i>D. marginatus</i>	—	+	—	128	—	+	—	> 128
Šnaberd (Artik)	<i>D. marginatus</i>	+	—	—	> 128	+	+	—	> 128
Šurabad (Amasija)	<i>D. marginatus</i>	+	—	—	n.d. ^b	—	+	16	8–64
Samagar (Ečmiadzin)	<i>D. marginatus</i>	—	—	—	64	+	+	—	64

^a Reciprocals of initial serum dilution.

^b Not done because of the early death of the animal.

Table 4. Rickettsial antibodies in human and animal sera collected in the Armenian SSR in 1971 and 1974

Serum	No. of sera examined in 1971	No. and proportion (%) of positive sera with antigen of:		No. of sera examined in 1974	No. and proportion (%) of positive sera with antigen of:	
		<i>C. burnetii</i>	spotted fever group		<i>C. burnetii</i>	spotted fever group
human	688	2 (0.3)	10 (1.5)	746	42 ^a (5.6)	79 ^b (10.6)
cattle	97	1 (1.0)	0 (0)	185	46 ^a (24.9)	26 ^b (14.1)
sheep	295	0 (0)	0 (0)	172	25 ^a (14.5)	15 ^b (8.7)
<i>M. arvalis</i>	165	0 (0)	0 (0)	15	1 ^a (6.7)	5 ^a (33.3)
<i>Mus musculus</i>				31	1 ^a (3.2)	5 ^a (16.1)

^a With microagglutination test.

^b With complement fixation test.

sera and the sera of small rodents, tested in parallel, were negative in the microagglutination test with stained *R. canada* antigen.

DISCUSSION

It is evident, from the data on the presence of spotted fever group rickettsiae in ticks and small mammals in regions of the Armenian SSR varying in altitude from 700 m to 2500 m, that these rickettsiae possess a marked ability to adapt to various habitats. This applies also to the rickettsiae of the spotted

fever group isolated in Czechoslovakia (1) and in Pakistan (16, 17), the conditions for the circulation of rickettsiae in Pakistan being similar to those in Armenia. The typical feature of all the natural foci in Europe and Asia where rickettsiae of the spotted fever group have been isolated is the heavy infection of ticks of the *Dermacentor* genus with these rickettsiae. Ticks of this genus are among the most common parasites of small wild mammals and of domestic animals, and thus may be the most important reservoirs and vectors of spotted fever group rickettsiae in the Armenian SSR. This is confirmed also by

the high incidence of antibodies to such rickettsiae in *Microtus arvalis* trapped at an altitude of 1500 m in one mountainous desert region of Armenia. Similar data on the infection of ticks and wild rodents with rickettsiae of the spotted fever group were obtained in Czechoslovakia (15). All the rickettsial strains from Armenia were isolated from ticks of the *Dermacentor* genus, namely, *D. marginatus*. None was isolated from *Haemaphysalis sulcata*. However, because our material was collected only in autumn, it is not excluded that other tick species and genera occurring in other seasons may play an important part in the ecology of spotted fever group rickettsiae.

Serological examination of healthy human beings, domestic animals, and small wild mammals showed differences in the prevalence of spotted fever group rickettsiae in Armenia between 1971 and 1974. These differences can be explained to some extent by the different antigens and serological tests used. The antigen used in 1971 was prepared from the *R. siberica* strain Necvetajev, which had been passaged more than one hundred times in chick embryos; that used in 1974 was prepared from a freshly isolated strain of spotted fever group rickettsiae (15). Though both antigens were soluble, their differing passage history under laboratory conditions may have led to differing serological reactivity. Furthermore, in 1971, the human sera were collected mostly among town dwellers who

had had no contact with natural foci, and the animal sera were taken in Ararat valley from domestic animals that had been kept free of ticks by means of regular checks. It must be remembered also that variations occur in the natural foci, e.g., in the degree of infection of ticks with rickettsiae and in the incidence of rickettsial antibodies in wild animals—as found by long-term observation of natural foci of Rocky Mountain spotted fever (2, 3, 13).

There remains the serious and unsolved problem of the proper identification and species characterization of the rickettsial strains isolated. Preliminary experiments have shown that those of the spotted fever group possess a broad spectrum of virulence for laboratory animals (M. Župančičová & V. A. Makarova, unpublished observations, 1973). Further investigation of the biological properties of these rickettsial isolates, especially their antigenicity and immunogenicity, is necessary.

Serological classification of isolates from Pakistan and Thailand (16) revealed that *Rickettsia siberica*, *R. conorii*, and a new, so far unclassified, rickettsia of the spotted fever group occur on the Indian subcontinent. Similarly, *R. siberica* and a rickettsial agent different from *R. siberica*, named *R. deserti pavlovskij* (12), were isolated in Turkmenistan, which is close to Armenia. On the basis of our results and of the previous ecological and clinical findings, it may be assumed that various rickettsiae of the spotted fever group may occur in the Armenian SSR.

RÉSUMÉ

ÉTUDES SUR LES RICKETTSIOSES.

1. FOYERS NATURELS DE RICKETTSIOSES EN RÉPUBLIQUE SOCIALISTE SOVIÉTIQUE ARMÉNIENNE

On a recherché, par l'épreuve des hémocytes et par des tests d'isolement, la présence de rickettsies dans des tiques ixodes, recueillies dans la République socialiste soviétique arménienne en 1971 et 1974. L'épreuve des hémocytes, effectuée par la méthode de coloration de Giménez, a révélé la présence de rickettsies du groupe de la fièvre pourprée ou d'organismes apparentés dans 163 tiques sur 894 (18,2%). Sur les 163 tiques positives, 2,6% l'étaient pour *Coxiella burnetii*, et 11,7% pour les rickettsies du groupe de la fièvre pourprée, ainsi que l'ont montré les méthodes d'immunofluorescence. Au total, 39 souches de rickettsies du groupe de la fièvre pourprée ont été isolées, soit 37 souches à partir de *Dermacentor marginatus* et deux à partir de *D. reticulatus*. Des tiques de ces deux espèces pouvaient transmettre les rickettsies à des cobayes, par morsure, et ont éliminé des rickettsies dans leurs déjections.

Des sérums humains, collectés dans les mêmes régions en 1971, contenaient des anticorps contre les rickettsies du groupe de la fièvre pourprée dans 1,5% des cas, et des anticorps contre *C. burnetii* dans 0,3% des cas; en 1974, les taux étaient respectivement de 10,6% et 5,6%. Des sérums collectés en 1974 sur des animaux domestiques et sauvages ont également réagi avec les rickettsies du groupe de la fièvre pourprée et avec *C. burnetii*: soit, respectivement, dans 14,1% et 24,8% des cas chez 185 vaches; 8,7% et 14,5% chez 172 moutons; 33,3% et 6,7% chez 15 *Microtus arvalis* et 16,1% et 3,2% chez 31 *Mus musculus*.

L'isolement des souches de rickettsies et la présence d'anticorps spécifiques dans les sérums humains et dans les sérums d'animaux domestiques et sauvages montrent qu'il existe en République socialiste soviétique arménienne des foyers naturels mixtes de rickettsioses du groupe de la fièvre pourprée et de la fièvre Q.

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