

NATURAL FOCALITY OF ZOONOTIC CUTANEOUS LEISHMANIASIS IN THE MONGOLIAN PEOPLE'S REPUBLIC; RESULTS AND OBJECTIVES OF INTEGRATED RESEARCH

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Abstract. In the summer 1976, natural foci of zoonotic cutaneous leishmaniasis were discovered by the authors on the territory of the Mongolian People's Republic. In 1976-1983 the integrated research of these foci was conducted and maps of a structure of the geographical range of the pathogen were compiled taking into account the peculiarities of distribution of its carriers and vectors. Great gerbils are the only identified vertebrate hosts of *Leishmania* and their infection rate comes up to 100 % in single foci. Three species of sandflies belonging to subgenus *Paraphlebotomus*, *Ph. andrejevi*, *Ph. mongolensis* and *Ph. alexandri*, are vectors of the pathogen in foci. The most common of them in burrows of gerbils is *Ph. andrejevi*. It was established by genetic and biochemical methods that two variants of *Leishmania major* may circulate simultaneously in individual natural foci in the MPR, but both of them have a low virulence and are not dangerous for man.

In the summer 1976, sandflies and epizootics of zoonotic cutaneous leishmaniasis (ZCL) among great gerbils were revealed at a few sites in the south of the Mongolian People's Republic (Neronov et al. 1977). In subsequent years, evidence was obtained on the range structure, ecology and exterior of the great gerbil, the principal host of the ZCL causative agent (Neronov and Lushekina 1980, 1981). Also data were processed on the sandfly fauna of Mongolia and its formation (Artemyev and Neronov 1982, 1984, Artemyev 1984); some new evidence was accumulated on natural ZCL foci and the structure of the Central-Asian part of the *Leishmania major* range (Neronov and Gunin 1978, Neronov et al. 1981). Within Central-Asia, three focal provinces have been delimited: East-Gobi-Alashan, Central-Gobi and Jungarian, covering not only the territory of the Mongolian People's Republic, but also the People's Republic of China and five autonomous focal districts: Shargin, Begernur, Oroknur, Bulgan, Bayandov, which lie in the MPR north of the main ZCL range. Until recently, no data have been available on the main properties of the causative agent circulating among great gerbils in the Mongolian People's Republic. Characteristically, no incidence of human cutaneous leishmaniasis has been recorded in the MPR or PRC (Wang et al. 1964a, b; Neronov and Gunin, 1978). This gave ground to Chinese researchers to regard the causative agent circulating among great gerbils in the adjacent foci of the PRC as a separate species, *Leishmania gerbilli* (Wang et al. 1964).

Along with that, the ZCL causative agent *Leishmania major*, circulating in different foci of the USSR has been reported to be highly variable with respect to virulence and ability to infect humans with Leishmaniasis (Kellina 1966). Moreover, these

indices may vary not only in different ZCL foci but in the same focus and even in the same great gerbil burrow (Lavrova et al. 1973, Malkhazova et al. 1977, Ponirovsky et al. 1980). Also, the ZCL ability to infect man has been found to vary considerably in the same focus during the transmission season (Strelkova et al. 1982). The above has led us to concentrate on the virulent properties and genetic and biochemical characters of *Leishmania* from natural foci in the MPR territory. In 1983 we isolated 10 *Leishmania* cultures from the great gerbils of two sites in the MPR. The present communication summarizes the results of this study. In addition, analysis has been made of the eight-year-long integrated research of the two other co-members of the ZCL parasitic system in the MPR, and objectives for further work have been set.

The causative agent

From 1976 to 1983, 416 great gerbils from 25 sites in the MPR were examined visually for skin lesions. Concurrently, smears were taken for microscopic investigation using the accepted technique of a cut along the upper edge of the floor of the auricle. 114 smears out of the 273 taken (41, 7 %) showed typical and rather numerous *Leishmania*. Examination of all the smears was performed by M. V. Strelkova of the Institute of Medical Parasitology and Tropical Medicine, Ministry of Health of the USSR. *Leishmania* were revealed both in adult individuals which overwintered and young from the current year's early litters. In the latter, skin lesions of the ears are less manifest or absent (Neronov et al. 1977, Neronov and Gunin 1978,

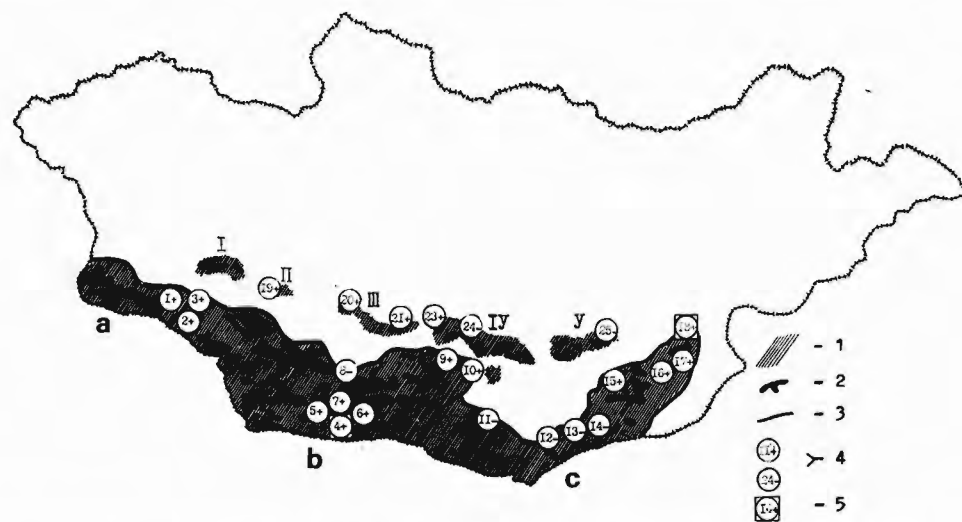


Fig. 1. Distribution of epizootics of zoonotic cutaneous leishmaniasis among great gerbils in the territory of the Mongolian People's Republic. 1 — area distribution of the great gerbil; 2 — sites with increased numbers of burrows of the great gerbil; 3 — borders of regional complexes of the autonomous population groups: A — Jungarian, B — Central-Gobi, C — East-Gobi-Alashan; autonomous population groups: I — Sharghin, II — Begernur, III — Oroknur, IV — Bulgan, V — Bayandov; 4 — sampling sites from where great gerbils were examined for the presence of leishmaniasis (+ — positive, — — negative); 5 — sites from where the causative agent's cultures were isolated.

Neronov et al. 1981). Thus, a total of 18 sites out of 25 were found to have epizootics among great gerbils (Fig. 1) both in the three major regional complexes of autonomous population groups and in the autonomous population groups (except Sharghin Gobi where no great gerbils were recorded by us over the last three years) north of the main range. The absence of infected great gerbils at certain sites (Nos. 8, 11–14) appears to be related to the inadequately small sample. The infection rate ranged from several per cent (Nos. 15–17) to 100 % (Nos. 21–23). On all occasions, leishmaniac lesions were only revealed in the floor of the auricle. The clinical feature of leishmaniasis ranged from asymptomatic to manifest thickenings at the edge of the floor of the auricle, accompanied by scaling of the affected skin areas, crusts, loss of hair and, occasionally, skin depigmentation. The examination of other rodent species (over 600 individuals) — the midday gerbil, clawed jird, several jerboa and hamster species — dwelling in the immediate vicinity or directly in the burrows of great gerbils has revealed no leishmaniasis. In August 1983, 10 *Leishmania* cultures were isolated from great gerbils: 8 in the vicinity of the somon Dzun-Bayan, East-Gobi Aimak (East-Gobi-Alashan focal province) and 2 in the Bayan-Khongor Aimak, near the settlement Eikhin-Gol (Central-Gobi focal province) (Fig. 1). The pathogen virulence was assessed in 1 to 3 passages after isolation, by infecting golden hamsters (subcutaneous injection into the floor of the auricle) and albino mongrel mice (subcutaneous injection into the supracaudal region). The times of the manifestation of the first clinical features, character and intensity of the process are the main distinctive features of high vs low virulence (Kellina et al. 1981). Man has been proved to fall ill and develop stable immunity only on having been infected with a highly virulent pathogen (Kellina et al. 1966). In experimental animals, a highly virulent pathogen produces rapidly developing infiltrates. After 4 to 8 weeks the latter ulcerate in golden hamsters, the ulcer size tending to increase progressively. The low-virulent pathogen cultures either do not cause illness in man or occasionally give rise to a brief abortive process, but no immunity develops. In laboratory animals such cultures cause illness only in some of the individuals. The process frequently involves a rather lengthy incubation period, without ever leading to ulceration. Often, the process is abortive and the animal recovers. All the leishmania cultures isolated in Mongolia showed low-virulent properties. Only some of the animals became infected, the incubation period ranging from 7 to 105 days. Leishmaniasis manifested itself as infiltrates of a variable size without a single instance of ulceration.

Six *Leishmania* cultures were tested using genetic and biochemical techniques: 2 (MPR-1 and MPR-2) from the neighbourhood of the Eikhin-Gol settlement and 4 (MPR-3, MPR-5, MPR-6, MPR-7) from the neighbourhood of the somon Dzun-Bayan. Depending on the number of suspensions of *Leishmania* cells, the number of the analyzed allozymic loci ranged from 8 to 10, including analysis of the enzymic systems as follows: malatdehydrogenase (MDH), phosphoglucomutase (PMG), phosphohexoisomerase (PHI), "malic" enzyme (ME), 6-phosphogluconatedehydrogenase (6PGD), glucoso-6-phosphatedehydrogenase (G6PDH), superoxydismutase (SOD), acid phosphotase (AP), aspartataminotransferase (ASAT), and esterase D (EstD). Also, isoelectric separation of the cytoplasm protein spectra was performed (IES). For comparison with the Mongolian isolates, the following reference cultures were used: MRHO (SU) 83 051 — *L. major*, isolated in spring 1983 from the great gerbil in the Mubarek District, Kashkadarya Region, Uzbek SSR; MHOM (SU) 82 — *L. major*, isolated in 1982 from a ZCL patient infected in the Turkmen SSR; MRHO (CN/00) *L. gerbilli*, isolated from the great gerbil in the PRC territory, time of isolation unknown; MHOM (IM/80) DD8 — *L. donovani*, isolated in 1980 from a patient in Bihar, India. The latter two cultures were obtained from the cryobank of the London School of Tropical Medicine and Hygiene and we are grateful to Prof. W. Peters for his valuable collaboration. The detail of the technique and results of these comparative studies are presented in a separate communication (Shurkhal et al., 1985). We shall consider here the principal conclusions from these experiments.

All the six *Leishmania* cultures from the MPR examined considerably differed genetically from the reference strain of *L. donovani*, and thus cannot be attributed to the group of the causative agents of visceral leishmaniasis. They all are the agents of cutaneous leishmaniasis (*L. major*) and can be divided into two genetically distinct groups. Group I, conventionally designated as "Turan-1" includes the isolates MPR-1, MPR-2, MPR-3 and the reference strain 051-*L. major* isolated from the great gerbil in the Uzbek SSR. Group II, conventionally designated as "Alashan" comprises the isolates MPR-5, MPR-6, MPR-7 and the reference strain *L. gerbilli* from the PRC. Importantly, both genetic variants may occur in great gerbils dwelling at the same site, in burrows situated in the immediate vicinity with respect to one another: the neighbourhood of the somon Dzun-Bayan, strain MPR-3 (Group "Turan-1") and strains MPR-5, 6, 7 (Group "Alashan"). The presence of the two genetically distinct varieties of the causative agent in the same great gerbil population, with transmission of the agent by a single vector species (*Phlebotomus andrejevi*) are indicative of a wide range of intraspecific heterogeneity in *L. major*. It is undoubtedly important to continue integrated studies of the genetic and biochemical indices of not only the pathogen — *L. major*, but also the great gerbil, the principal host, and sandflies, which are the vectors.

Leishmania vectors

From 1976 to 1983, a total of 22,323 sandflies were captured in the MPR. The material was treated by M. M. Artemyev (Institute of Medical Parasitology and Tropical Medicine, USSR Ministry of Health). Sandflies were sampled throughout all the ZCL foci. They were sampled mostly in great gerbil burrows, some of the catches were from the burrows of the midday gerbil and Palas's pika. The distribution of sampling sites is given in Fig. 2. Over the above mentioned period, 6 sandfly species were revealed in the MPR territory: *Phlebotomus* (*Paraphlebotomus*) *alexandri*,

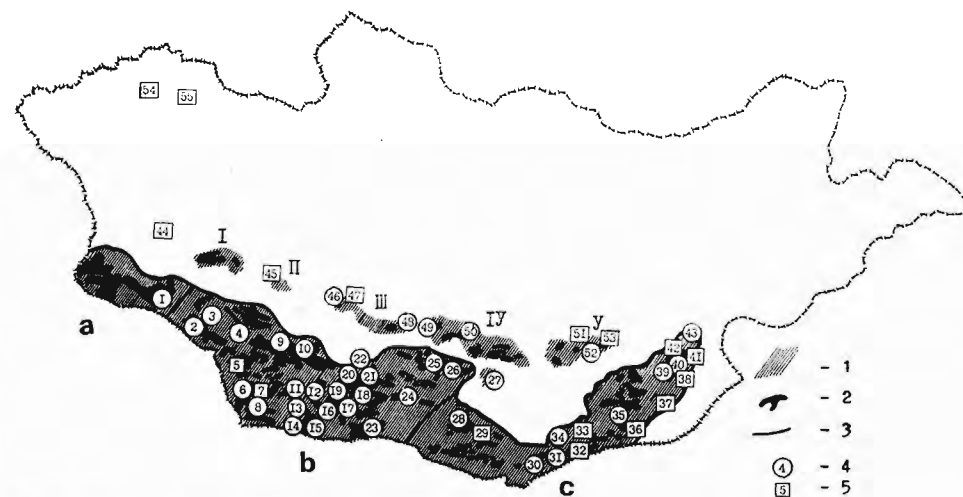


Fig. 2. Ecological and faunistic observations on sandflies in the territory of the Mongolian People's Republic. 1 — 3 as in Fig. 1: 4 — points showing sites sandflies were found; 5 — sites where sandflies were not recorded.

Ph. (P.) andrejevi, *Ph. (P.) mongolensis*, *Ph. (Larroussius) wui*, *Sergentomyia* (*Sergentomyia*) *murgabiensis murgabiensis*, *S. (Parrofomyia) gobica* (Artemyev and Neronov 1982, 1984, Artemyev 1984). The first two species are the vectors of the ZCL causative agent, *L. major*, while *Ph. wui* is the vector of the pathogen of visceral leishmaniasis. The sandflies of the genus *Sergentomyia* are of no medical consequence, since they are very rarely hosted by mammals, great gerbil including, and are closer related trophically to reptiles. 67 % of the captured sandflies are accounted for by *Ph. andrejevi*. This is readily understandable since most of the catches were made in sandy desert in great gerbil burrows, where this species predominates. 30 % of the captured individuals are *Ph. mongolensis* and *Ph. alexandri*, also members of the subgenus *Paraphlebotomus*, and only 3 % are the remaining three species. The greatest species diversity is recorded in the Central Gobi focal province, the central part of it being characterized by a mosaic of plain and mountain landscape. A single vector species was found in the two other focal provinces: *Ph. andrejevi* in the East-Gobi-Alashan and *Ph. mongolensis* in Jungarian.

To summarize the eight-year faunistic studies in the ZCL foci in the MPR, there is a reason to believe that the Mongolian sandfly fauna has been extensively studied and it can be hardly expected that 1—2 previously unrecorded species may be found in the near future. Analysis of the Mongolian sandfly fauna indicates that all the recent sandfly species have penetrated Gobi from the Middle Asia comparatively recently. Although *Ph. wui* and *S. gobica* have attained the rank of separate species, it is evident that they are closely related to the Middle-Asian *Ph. smirnovi* and *S. sumbarica*. The remaining sandfly groups represent the fauna of the Ancient Mediterranean and are too young to have evolved into separate species or even subspecies. In the years to come, it will be necessary to perform more long-term studies of different *L. major* vectors to reveal their breeding features as well as local and trophic relations to various hosts and the dynamic of various natural and human factors with a special reference to their impact on the ability to attack and transmit the causative agent to the man.

Vertebrate hosts of *Leishmania*

In the section "The causative agent" we have already noted that the principal host of *L. major* in the MPR territory is the great gerbil. Occasionally, the whole populations are infected. This has led us to focus our attention on the distribution pattern and intraspecific structure of the rodent.

The first great gerbil finds in Mongolia were described by N. M. Przevalsky in 1883 (see Przevalsky 1948). Analysis of published data and his own observations permitted Bannikov (1954) to design a detailed map of this species range, showing 21 sites of great gerbil finds. According to Bannikov (1954), its smallest subspecies, *R. o. nigrescens* Satunin, 1902, is distributed ubiquitously, while in other regions of the Central Asia, there occur bigger subspecies, different in coloration — *R. o. pezvovi* (south of Jungaria and Kashgaria), *R. o. giganteus* (North Jungaria) and *R. o. alashanicus* (Alashan).

Over 8 years we conducted the route observations (about 90 thousand kilometers by car along with numerous walking routes) of a variety of habitats of the great gerbil in the MPR. Analysis of our findings, using soil and vegetation maps, has resulted in specifying the range and delimiting in the MPR three regional complexes of autonomous population groups: Jungarian, Central-Gobi, East-Gobi-Alashan and five autonomous population groups north of the major range: Sharghin, Begernur, Oroknur, Bulgan and Bayanov (Neronov and Lushekina 1980) (see Figs. 1 and 2). Examination

of these regions revealed that the densest gerbil colonies were in the thickets of black saxaul, bottoms of lake depressions, piedmont area of unplands and cobbly desert. Along sandy sairs (often free from saxaul), some burrows are found at an altitude of up to 1,900—2,000 m asl (ridges Tzagan-Bodgo, Azdh-Bodgo, south of Alag-nur). The greatest burrows with pronounced "ecological centres", a network of subterranean patches and "new settlements", often at a distance of 40—50 m from the centre, are characteristic of the Jungarian regional complex. In the optimal part of this complex, 7—11 inhabited burrows were recorded per 1 km of vehicle route in cobbly desert, with low saxaul stands (east of the spring Khonin-Us) in August 1980. In 1955—1956 a drop in gerbil numbers was noted there, with most burrows (4—5 per 1 km) abandoned (Chugunov 1962). Over the last three years, we have never found great gerbils in Sharghin-Gobi where their colonies were described by Bannikov (1954) and Tarasov (1958). In the north-east and east of the range in the summer 1980, the great gerbil numbers likewise sharply declined.

In the summer seasons of 1980, 1982, 1983, we (Neronov et al., in press) took 296 adult great gerbils from 13 sites to study the variability of their exterior characters. This study demonstrated that with pronounced sex dimorphism (males are much bigger than females), the highest average measurements (weight and length of body, tail, hind foot) are characteristic of the gerbils of the autonomous population groups (Oroknur, Bulgan and Bayandov) north of the main range. In the western part of the main range (Jungarian regional population complex), the gerbils are somewhat bigger (both in weight and body length), but in other indices, they are inferior to the animals from the East-Gobi-Alashan regional complex. Our data invariably differ from the measurements given by Bannikov (1954), but Bannikov sampled the gerbils without differentiating the sexes, which we believe to be incorrect.

The highest percentage of positive smears (80—100 %) for leishmaniasis infection was accounted for by the samples of great gerbils from the Oroknur and Bulgan autonomous population groups. Possibly, further accumulation of representative evidence will reveal whether there is a relationship between the exterior characters of certain great gerbil populations and their leishmaniasis resistance. To elucidate the reasons for the above differences in the exterior characters of different populations of the great gerbil, further eco-geographical studies of its species population are required, involving data on the genetic proximity of the populations compared. Similar to the study of the causative agent (see above), the electrophoretic method may prove to be very useful here.

CONCLUSIONS

The analysis of data obtained in the course of eighty-year-long studies on ZCL natural focality in the MPR revealed that the great gerbil, sandflies *Ph. andrejevi*, *Ph. mongolensis* and *Ph. alexandri*, as well as *L. major*, form a single parasite-host complex, which penetrated into the MPR territory from the Turan-Kazakh part of the ZCL range. The entire complex is relatively new to Mongolia. The natural foci developed there after the great gerbil penetrated from the Middle Asia through the Jungarian and Tarym plains and were apparently formed during the interglacial and postglacial periods (Murzaev 1966). The great gerbil is the main host of *L. major* through a vast area within the bounds of the Soviet Union, where this species was first described by Yakimov (1915), and also in Iran, Afghanistan and Pakistan. Only the eastern part of the range of this widely distributed species extends to

Mongolia and China. At the present time, the Iran-Turanian and Central-Asian parts of the ZCL range are isolated.

The Iran-Turanian deserts differ from the Central-Asiatic in a number of essential nature characteristics: amount and distribution pattern of precipitation seasonally, temperature amplitude, duration of warm and cold seasons (Petrov 1973). But the differences in the nature conditions of the deserts of Turan and Central Asia have had no essential effect on the isolation of the populations of the great gerbil, sandfly and *Leishmania*. Still the absence in the Central Asia of *Ph. papatasi*, an extremely effective vector of *L. major*, should be taken into account. Nevertheless, the presence in the great gerbil population of Mongolia of the genetic variety of *Leishmania* identical to the causative agent in the ZCL foci of the USSR also supports the same origin of the parasite-host complex of the Iran-Turanian and Central-Asian focal provinces.

Studies on the virulent properties of the causative agent circulating in the MPR foci among great gerbils demonstrate that the MPR's natural foci do not contain the epidemically hazardous highly virulent variety of the causative agent. The latter appears to account for the fact that the natural foci of the ZCL in Mongolia do not manifest themselves epidemiologically. Similar data are reported for the ZCL foci in the People's Republic of China (Wang et al. 1964a, c). The absence of other leishmaniasis infected wild mammals dwelling jointly with the great gerbil (midday gerbil, clawed jird, jerboas, etc.) which, similar to man, contract infection from a highly virulent pathogen only, is also in agreement with the absence in the pathogen's population of highly virulent variety. It should be borne in mind, however, that a lowly virulent pathogen penetrating human skin occasionally may produce skin lesions, which do not lead to a clinical manifestation of the disease and development of immunity. Along with that, the ability to infect the man and animals other than the great gerbil is not an obligate trait of all the *L. major* populations in the Middle-Asiatic part of this parasite's geographical range either.

The natural ZCL foci with high infection rates for the great gerbil and no incidence of human infection recorded are in the northern deserts of the USSR (situated in the same latitude as the ZCL foci in Mongolia), and also in some local areas of the southern deserts immediately bordering on epidemically active natural foci. ZCL natural foci are characterized by long-term and seasonal variability of epidemiological activity. Thus, data available to date indicate that the maintenance of a natural focus does not necessarily require a highly virulent variety of the pathogen. The transition of a part of or entire population of *L. major* into a highly virulent state, with the pathogen acquiring the ability to infect the man, is not invariable, but depends on some special conditions of the pathogen's circulation. As shown by the research conducted in the ZCL foci in the USSR, the transformation of the pathogen's virulent properties was noted more frequently where transmission involved *Ph. papatasi*, and was characterized by a high intensity, a function of vector numbers and duration of transmission period. During winter, with a pathogen not transmitted via vectors, the causative agent, which dwelled in the great gerbils ears is transformed into the initial low virulent state (Strelkova et al. 1982). Apparently, the shorter period of the pathogen's transmission in the ZCL foci in Mongolia, as determined by the specificity of Mongolian desert climate, restricts the lifespan of sandfly females, reducing, thereby, the number of pathogen transmissions. The latter is not conducive to the transformation of the agent's virulent properties. Also essential is the absence in the MPR foci of *Ph. papatasi*, providing conditions for selection of highly virulent *Leishmania* varieties even within a single gonotrophic cycle (Strelkova et al. 1981).

The above data prove unsubstantiated the classification of the parasites circulating in the Central Asian focal provinces as separate species, as proposed by the Chinese

workers. We believe that the great gerbil population of these focal provinces hosts the lowly virulent variety of *L. major* of no epidemiological importance.

ПРИРОДНАЯ ОЧАГОВЫЙ ЗООНОЗНОГО КОЖНОГО ЛЕЙШМАНИОЗА НА ТЕРРИТОРИИ МНР: ИТОГИ И ЗАДАЧИ КОМПЛЕКСНЫХ ИССЛЕДОВАНИЙ

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Резюме. Летом 1976 г. на территории Монгольской Народной Республики авторами впервые обнаружены природные очаги зоонозного кожного лейшманиоза. С 1976 по 1983 г. проведены комплексные исследования этих очагов на всей территории МНР и составлены карты структуры ареала возбудителя с учетом особенностей распространения его носителей и переносчиков. Единственным выявленным позвоночным хозяином лейшманий служат большие песчанки, зараженность которых в отдельных очагах достигает 100 %. Переносчиками являются три вида moskitov подрода *Paraphlebotomus* — *Ph. andrejevi*, *Ph. mongolensis*, *Ph. alexandri*. Наиболее обычен из них в норах большой песчанки *Ph. andrejevi*. Генетико-биохимическими исследованиями установлено, что в отдельных природных очагах в МНР могут циркулировать сразу два варианта *Leishmania major*, но оба они слабовирусulentны и не опасны для человека.

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Received 13 September 1984

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