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The role of *Phlebotomus alexandri* Sinton, 1928 in the transmission of kala-azar*

GUAN LI-REN,[1] XU YONG-XIANG,[2] LI BAO-SHAN,[3] & DONG JIANG[4]

Since 1968, kala-azar has been occurring sporadically in Meiyaogou, Turfan County, Xinjiang-Uygur Autonomous Region, where four species of sandflies are known to exist. The present study of sandflies collected in this area from May to August 1983 shows that Phlebotomus alexandri *is the only anthropophilic and predominant species, accounting for 81.1% (7716/8843) of the sandfly population. After having been fed on cotton rats or hamsters infected with* Leishmania donovani, *93.9% (230/245) of* P. alexandri *were infected, the promastigotes not only developing in the stomach but also invading the pharynx, buccal cavity and proboscis. Of the 643 female* P. alexandri *collected in the wild and in houses, 13 (2.0%) were found to be naturally infected, with a distribution of promastigotes similar to that in the experimental infection. All the Chinese hamsters and golden hamsters that had been inoculated with 7 isolates of promastigotes from naturally infected sandflies developed visceral leishmaniasis. In addition, golden hamsters could be infected experimentally by the bite of* P. alexandri *which had an infection of promastigotes of* L. donovani. *This is the first demonstration of a species of* Paraphlebotomus *playing a role in the transmission of kala-azar in the Old World; studies for confirmation by biochemical identification of parasite isolates from naturally infected* P. alexandri *are now in progress.*

Since 1968, cases of kala-azar have been occurring sporadically in Meiyaogou (in Turfan County) in the Xinjiang (Sinkiang)-Uygur Autonomous Region of China. While the insect vectors *Phlebotomus alexandri* Sinton, 1928 and *Sergentomyia minutus sinkiangensis* Ting & Ho, 1962 were found in this area in 1973 (*1*) and a new species, *S. turfanensis* Xiong, Guan & Jin, 1981, was reported in 1975 (*2*), the three known vectors of kala-azar in China (*P. chinensis* Newstead, 1916, *P. chinensis longiductus* Nitzulescu 1931, and *P. major wui* Yang & Xiong, 1965) were not found. It was inferred that *P. alexandri* might be the vector of kala-azar in the study area (*1*) and the results of investigations carried out in 1983 on this possibility are described below.

MATERIALS AND METHODS

Leishmanin test and endemicity of kala-azar in the area

The intradermal test (with leishmanin as antigen, prepared in our Institute) was carried out on the Uygur population and on immigrants from non-endemic regions between 1966 and 1983; case histories of kala-azar were reviewed to establish the present status and any endemic tendency of kala-azar.

Distribution of various sandfly species

Sandflies were collected extensively with tube aspirators or sticky paper for identification.

Sandfly behaviour in relation to animals and man

Female sandflies were collected before blood-sucking and kept in a cage. An anaesthetized hamster

* From the Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, WHO Collaborating Centre for Malaria, Schistosomiasis and Filariasis, 207 Rui Jin Er Lu, Shanghai, China. The original version of this paper (in Chinese) was published in the Chinese *Journal of Parasitology and Parasitic Diseases*. This English version is published here with kind permission of the Editor of that journal.

[1] Assistant Research Fellow, Department of Epidemiology, Institute of Parasitic Diseases. Requests for reprints should be sent to this author.

[2] Senior Technician, Department of Epidemiology, Institute of Parasitic Diseases.

[3] Chief, Parasite Section, Anti-Epidemic Station, Urumqi, Xinjiang-Uygur Autonomous Region, China.

[4] Physician, Parasite Section, Anti-Epidemic Station, Urumqi, Xinjiang-Uygur Autonomous Region, China.

(homothermic) or lizard (poikilothermic) was put into the cage and exposed to the bite of the sandflies. After 2 to 6 hours, the sandflies were identified and classified as anthropophilic or cold-blooded feeders. These data were supplemented by taxonomic identification of the sandflies collected from human dwellings and from the bodies of the occupants.

Artificial infection of P. alexandri

P. alexandri sandflies were collected at dusk from the field by means of human baits and introduced immediately into a cage. Cotton rats (*Sigmodon hispidus*) or golden hamsters (*Mesocricetus auratus*), infected with the Xinjiang strain of *Leishmania donovani*, were exposed to these sandflies. The animals had been anaesthetized by intraperitoneal injection of urethane and their abdominal skins were well shaven before exposure. They were taken out of the cage after 2 hours and those sandflies that were engorged with fresh blood were transferred the next morning into glass tubes with plaster at the bottom, fed 5% glucose solution, and kept at room temperature (26–28 °C). Batches of these sandflies were dissected daily from the 4th day onwards to observe the growth and development of promastigotes of *L. donovani* in their alimentary tracts.

Natural infection of P. alexandri

Sandflies were collected from the field or households and those with their blood meals partially or completely digested were dissected and examined for signs of natural infection (promastigotes) in their alimentary tract. Promastigotes, when present, were isolated and inoculated into the peritoneal cavity and skin of normal hamsters (*M. auratus* and *Cricetulus barabensis*). About 3 months later, the hamsters were sacrificed and examined for amastigotes and their localization.

Experimental transmission of L. donovani *to normal hamsters through the bite of* P. alexandri

P. alexandri, after being fed on cotton rats infected with *L. donovani*, were kept for 11–12 days and released into a cage; an anaesthetized uninfected golden hamster was then introduced and kept in the cage overnight. The sandflies were examined for blood-taking and infectivity the next day, while golden hamsters that had been exposed to their bites were dissected 5 months later and examined microscopically for signs of visceral leishmaniasis.

RESULTS

Leishmanin test and endemicity of kala-azar in the area

The intradermal test with leishmanin was performed on the local population in Meiyaogou and the proportion of positive reactions appeared to vary with the duration of residence in the area (Table 1). Among infants who had experienced only one sandfly season, 6 out of 17 showed a positive reaction. The positive rate of this test in the Uygur population was significantly higher than that in the immigrant Han population, owing to the outdoor sleeping habits among the Uygur during the sandfly season. Two autochthonous cases of kala-azar occurred in this area between October 1982 and February 1983: one was a 2½-year-old Uygur child and the other a Han child aged 6 years and 3 months. Both had never been out of this area and were cured by treatment with sodium stibogluconate.

Distribution of various sandfly species

A total of 8843 sandflies were collected from Meiyaogou and identified. Of the four species dis-

Table 1. Results of the leishmanin test on the local population (Uygur and Han) in Meiyaogou, by the duration of residence in the area

No. of years of residence	Uygur population		Han population		χ^2 test (*P* value)
	No. tested	No. positive	No. tested	No. positive	
1–5	21	7 (33.3)[a]	59	7 (11.9)	<0.05
6–10	48	31 (64.6)	145	20 (13.8)	<0.001
11–15	55	36 (65.5)	90	29 (32.2)	<0.001
16	10	9 (90.0)	8	3 (37.5)	<0.05
Total	134	83 (61.9)	302	59 (19.5)	<0.001

[a] Figures in parentheses are percentages.

Table 2. Distribution of various sandfly species in Meiyaogou

Locality	Total number of sandflies captured	*P. alexandri*		*P. mongolensis*		*S. turfanensis*		*S. minutus sinkiangensis*	
		No.	%	No.	%	No.	%	No.	%
Wild	6914	5386	77.9	11	0.2	1141	16.5	376	5.4
Houses	1929	1790	92.8	8	0.4	103	5.3	28	1.5
Total	8843	7176	81.1	19	0.2	1244	14.1	404	4.6

covered, two belonged to *Phlebotomus* (subgenus *Paraphlebotomus*) — *P. alexandri* and *P. mongolensis* — and two to *Sergentomyia*, namely *S. turfanensis* and *S. minutus sinkiangensis*. *P. alexandri* was the predominant species, accounting for 81.1% of the sandflies captured (Table 2).

In Potaogou, a non-endemic area, 138 sandflies were caught on four occasions. They comprised 67.4% *S. minutus sinkiangensis*, 25.4% *P. mongolensis*, and only 7.2% *P. alexandri*. The composition of the sandfly population here was thus very different from that in Meiyaogou.

Sandfly behaviour in relation to animals and man

When kept in a cage with one hamster or two lizards, *P. alexandri* was found to prefer the hamster (blood-sucking rate, 79.0%) and to refuse the lizard's blood. On the other hand, *S. turfanensis* and *S. minutus sinkiangensis* fed only on the lizard's blood (Table 3). Both species of *Sergentomyia* were ecologically associated with the lizard (*Alsophylax microtis*) in nature, and nucleated lizard erythrocytes were often found in their stomach. From June to August, out of a total of 837 sandflies collected by human baits in the field at dusk, 835 (99.8%) were *P. alexandri* and only 2 (0.2%) were *S. turfanensis*. Of the 1929 sandflies caught from households, *P. alexandri* accounted for 92.8% (Table 2). Forty-two (80.8%) blood-meal samples out of 52 obtained from *P. alexandri* had human blood, as identified by the precipitation test: 29 showed a positive reaction with human antiserum, 13 with antisera from man and sheep, and 1 with antiserum from sheep, 9 samples being negative with the above-mentioned antisera.

The results indicate that *P. alexandri* is an anthropophilic species of epidemiological significance in the transmission of kala-azar in the study area; the role of *S. minutus sinkiangensis* and *S. turfanensis* as vectors of human leishmaniasis can be ruled out owing to their preference for lizard's blood.

Artificial infection of P. alexandri

P. alexandri sandflies were dissected between the 4th and 11th days after exposure to cotton rats or hamsters infected with the Xinjiang strain of *L. donovani* and the result showed a high infection rate among the sandflies (around 90%) (Table 4). The promastigotes persisted after the digestion of the blood meal in the stomach of the sandflies. Thus, *P. alexandri* appears to be a favourable host for the development of *L. donovani* at 4–5 days after feeding, the promastigotes being often attached to the stomach wall of the sandfly by the tip of their flagella. On the 6th to 7th day, the blood meal had been completely digested in many sandflies and the stomach and proventriculus were filled with promastigotes, which then invaded the oesophagus and

Table 3. Feeding habit of different species of sandflies

Animal	*P. alexandri*		*S. turfanensis*		*S. minutus sinkiangensis*	
	No. exposed	No. fed	No. exposed	No. fed	No. exposed	No. fed
Hamster	400	316 (79.0)[a]	32	0	21	0
Lizard	96	0	35	34 (91.7)	27	27 (100)

[a] Figures in parentheses are percentages.

Table 4. Artificial infection of *P. alexandri* with *L. donovani*

Days after feeding	No. dissected	No. infected	Distribution of promastigotes in sandflies						
			Proboscis	Buccal cavity	Pharynx	Esophagus	Proventriculus	Midgut	Hindgut
4	2	2	0	0	0	0	1	2	0
5	15	13 (86.7)[a]	0	0	0	6	13	13	3
6	22	21 (95.5)	0	0	1 (4.8)	12	21	21	1
7	27	25 (92.6)	0	0	3 (12.0)	19	25	25	2
8	47	44 (93.6)	0	0	12 (27.3)	36	44	44	2
9	43	39 (90.7)	2	1	13 (33.3)	33	39	39	3
10	50	49 (98.0)	4	0	19 (38.8)	44	49	49	0
11	39	37 (94.9)	6	2	19 (51.4)	31	37	37	2
Total	245	230 (93.9)							

[a] Figures in parentheses are percentages.

pharynx. The rate of pharygeal infection increased with time. Rosette formation of the promastigotes was also frequently seen. On the 9th day, the promastigotes had migrated to the proboscis (Table 4). In some heavily infected sandflies, a few rosette-like clusters of promastigotes were found at the junction of the Malpighian tubules and the hindgut, but the rectum was not involved. It was demonstrated that the promastigotes of *L. donovani* migrated forwards in the digestive tract of *P. alexandri*, but were not discharged from the anal cave.

Natural infection of P. alexandri

A total of 643 female *P. alexandri* sandflies were collected from different places and examined; 13 (2.0%) were found to be infected with promastigotes. Among these, 12 had completely digested their blood meal, and 6 of them were heavily infected with promastigotes which flocked in the stomach and invaded the pharynx and even the buccal cavity and proboscis (Table 5).

Seven isolates of promastigotes obtained from naturally infected *P. alexandri* were inoculated intraperitoneally and subcutaneously into normal hamsters which developed visceral leishmaniasis later. The promastigotes found in naturally infected *P. alexandri* can tentatively be considered to belong to *L. donovani* (Table 6), pending biochemical characterization of the isolates from this sandfly.

The monthly natural infection rate of *P. alexandri* was observed from May to August. No infection was found in May (0/13), the first infection appeared on 25 June, and the infection rates in June, July and August were 0.5% (1/213), 2.5% (4/160) and 3.1% (8/257), respectively. This shows that the risk of kala-azar transmission began at the end of June, with July and August as the major transmission seasons in this area. In addition, the examination of 58 *S. turfanensis* and 30 *S. minutus sinkiangensis* female sandflies showed no promastigotes in the stomach. Heart blood and liver tissue from 17 lizards were cultivated in NNN (Novy-Nicolle-MacNeal) culture medium and showed no growth of *Leishmania*.

Experimental transmission of L. donovani *to normal hamsters through the bite of* P. alexandri

Two batches of 18 and 23 sandflies were fed on a cotton rat that had been infected with *L. donovani*.

Table 5. Distribution of promastigotes found in naturally infected *P. alexandri*

Locality of collection	No. dissected	No. infected	Proboscis	Buccal cavity	Pharynx	Esophagus	Proventriculus	Midgut
Dry gully	262	8 (3.1)[a]	1	1	3	4	7	8
Wild caves	154	4 (2.6)	0	0	2	3	4	4
Human dwellings	227	1 (0.4)	0	0	1	1	1	1

[a] Figures in parentheses are percentages.

Table 6. Result of inoculation of hamsters with promastigotes from naturally infected *P. alexandri*

Sandfly No.	Experimental animal	Days from inoculation to dissection	Amastigotes in hamster				
			Spleen	Liver	Bone-marrow	Lymph-node	Subcutaneous tissue
1	*Cricetulus barabensis*	97	+	+	+	+	–
2	*C. barabensis*	88	+	+	+	+	–
6	*Mesocricetus auratus*	86	+	+	+	–	–
7	*C. barabensis*	88	+	+	+	+	+
10	*M. auratus*	90	+	–	–	–	–
11	*C. barabensis*	93	+	+	–	–	–
13	*C. barabensis*	93	+	+	+	+	+

After 11 and 12 days, respectively, two normal golden hamsters were exposed to their bite. Only 3 sandflies took blood from No. 1 hamster and 7 from No. 2. As these sandflies were gravid, the blood volume engorged corresponded only to a third to a half of their abdomen's capacity. All the 10 sandflies containing fresh blood were dissected and promastigotes were found in 6 of them. Except for one sandfly which had been fed on hamster No. 1 and showed light infection with promastigotes in the midgut only, the other 5 sandflies were heavily infected with promastigotes in the stomach, proventricules and pharynx, and even in the proboscis (in one of them).

Hamster No. 1 was dissected on day 147 after having been bitten by artificially infected *P. alexandri* and hamster No. 2 on day 145. Splenomegaly developed in hamster No. 1 and hepatomegaly in No. 2. Smears of liver, spleen, bone-marrow, lymphnodes, and subcutaneous tissue were prepared. Amastigotes could be found on Giemsa-stained smears of the liver and spleen from both animals and in the lymphnodes of hamster No. 1. This result shows that the golden hamster could get visceral leishmaniasis through the bite of *P. alexandri* which had been infected with promastigotes of *L. donovani*.

DISCUSSION

In 1957 it was reported that members of the *P. major* group were responsible for kala-azar in the Old World (*3*), and a few years later species of the "Synphlebotomus" complex (closely related to the *P. major* group) were reported to be vectors of the disease in East Africa (*4*, *5*). In general, species of the subgenus *Paraphlebotomus* were incriminated as vectors for the transmission of cutaneous leishmaniasis only. For instance, based on epidemiological data, the role of *P. alexandri* in the transmission of cutaneous leishmaniasis was recognized in certain regions of Central Asia (USSR) and Turkey (*6*, *7*), and its natural infection with an unidentified species of *Leishmania* in Khuzestan province of Iran has also been reported (*8*). In the area of Heishanhu of Jiuquan County, Gansu province of China, where *L. gerbilli* was present, natural infection with flagellates was observed in *P. alexandri* which was considered to be a vector of gerbil leishmaniasis (*9*). In the same area, in *P. alexandri* sandflies that had experimentally been infected through the biting of hamsters parasitized with *L. donovani*, it was reported that promastigotes developed well in the stomach and could invade the pharynx and, occasionally, the proboscis; this species of sandfly was therefore considered to possess the characteristics essential for the transmission of kala-azar (*10*). In the present study, *P. alexandri* has for the first time been shown to be a vector of kala-azar in the Old World by field observations and laboratory experimentation.

The region of Meiyaogou occupies a gully formed by running water from the Tianshan mountain cutting the stone desert at the foot of the mountain, the banks of the gully being rocky precipices. This is an isolated area far from the old endemic foci of kala-azar. Before 1966, only at the end of the gully were there a few residents of Uygur nationality. With the development of this area, both Uygur and Han populations gradually moved in. In 1968, the first case of autochthonous infantile kala-azar occurred in an 8-month-old Han child. Thereafter, 1–8 cases of kala-azar occurred every year after the sandfly season. Man became infected with kala-azar after entering these originally desert areas for production

and other activities. In 1983, only two baby dogs were identified which had not experienced the sandfly season; they were therefore not likely to be the source of natural infection of *P. alexandri*. It is presumed that *L. donovani* existed in this area long before its occupation by people, probably in wild animals yet to be discovered. Further studies are needed to elucidate the existence of a natural reservoir from an epidemiological point of view.

In 1974 another focus of kala-azar in some newly established farms on the borders of the Takelamagan desert in Xinjiang was reported (*11*). The sole local sandfly was the wild species *P. major wui*, and the natural infection rate of promastigotes of *L. donovani* during the sandfly season was 2.9%. Feeding on hamsters infected with *L. donovani*, 85.1% of *P. major wui* were infected and the promastigotes could invade the pharynx. It was considered that this species of sandfly was the vector of kala-azar in this dry desert area (*11*). However, in the very different stony desert area of Meiyaogou *P. alexandri* was clearly the most predominant species and *P. major wui* was absent. These results indicate that sandflies of different species may be active in kala-azar transmission in different regions, which needs to be confirmed by further study.

In conclusion, the present findings on *P. alexandri* meet most of the criteria for vector incrimination laid down by the WHO Expert Committee on Leishmaniasis (*12*); studies on the biochemical identification of parasite isolates from this sandfly are now being pursued for confirmation.

RÉSUMÉ

LE RÔLE DE *PHLEBOTOMUS ALEXANDRI* SINTON, 1928 DANS LA TRANSMISSION DU KALA-AZAR

Depuis 1968 des cas sporadiques de kala-azar s'observent au Meiyaogou, district de Turfan, Région Autonome Ouigoure du Xinjiang. Quatre espèces de phlébotomes, à savoir *Phlebotomus alexandri* Sinton, 1928, *P. mongolensis* Sinton, 1928, *Sergentomyia turfanensis* Xiong, Guan et Jin, 1981 et *S. minutus sinkiangensis* Ting et Ho, 1962 ont été capturées dans cette région du mois de mai au mois d'août 1983. *P. alexandri*, l'espèce prédominante, représente 81,1% (7716/8843) de la population totale de phlébotomes. C'est aussi la seule espèce anthropophile. Après s'être nourries sur des sigmodons ou des hamsters infestés par *Leishmania donovani*, 93,9% (230/245) des *P. alexandri* ont été infestés à leur tour et les promastigotes se sont développés non seulement dans l'estomac mais ont aussi enhavi le pharynx, la cavité buccale et la trompe. Parmi les 643 *P. alexandri* femelles collectées à l'extérieur et dans les habitations, 13 (2,0%) ont été trouvées infestées de promastigotes, avec une distribution analogue à celle de l'infestation expérimentale. Tous les hamsters chinois et les hamsters dorés inoculés avec 7 isolements de promastigotes provenant des phlébotomes naturellement infestés ont fait une leishmaniose viscérale. C'est la première fois que l'on démontre qu'une espèce du sous-genre *Paraphlebotomus* peut jouer un rôle dans la transmission du kala-azar dans l'ancien monde. Des études sont en cours en vue d'identifier du point de vue biochimique des isolements de parasites provenant de *P. alexandri* naturellement infestès.

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