Reprinted from Annals of Tropical Medicine and Parasitology, Vol. 73, No. 5 (1979)

Isolation of *Leishmania major* from *Mastomys erythroleucus* and *Tatera gambiana* in Senegal (West Africa)

By J.-P. DEDET and F. DEROUIN

Institut Pasteur, PO Box 220, Dakar, Senegal

B. HUBERT

ORSTOM, PO Box 1386, Dakar, Senegal

L. F. SCHNUR and M. L. CHANCE

Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, England

Received 15 August 1978

Cutaneous leishmaniasis was first described in Senegal by Riou and Advier in 1933, and its prevalence is known from the work of Larivière (1966). A recent increase in the disease has been reported by the dermatologists of the Dakar hospitals (Marchand, 1976, personal communication).

Following the discovery of infected *Arvicanthis niloticus* (Larivière *et al.*, 1965), Ranque *et al.* (1974) assumed that *Arvicanthis niloticus* is the main reservoir of cutaneous leishmaniasis in Senegal.

Since 1976 we have carried out an epidemiological survey of the disease in the Thies region of Senegal. In this region rodent burrows are a favoured resting site of *Phlebotomus duboscqi*, and two female *P. duboscqi* were found naturally infected with promastigotes (Dedet *et al.*, 1978). This paper presents the results of a search for the animal reservoir.

MATERIALS AND METHODS

Region Studied

The region consisted of a large, enclosed, cultivated area belonging to the Monastery of Keur Moussa, near the city of Thies, in the Cap-verdienne region, where cutaneous leishmaniasis is endemic. This region was chosen because of the numerous cases of cutaneous leishmaniasis in the monastery and the neighbouring villages, and because of the presence of numerous rodent burrows. Twenty human strains were isolated between December 1976 and October 1977.

Rodents

Rodents were caught live in steel wire traps, killed with ether, examined for superficial lesions and then necropsied. The species, sex and age (determined by the weight of the dried eye-lens, as described by Hubert and Adam, 1975) of each animal were recorded. Spleen, liver, blood and bone-marrow samples were inoculated into NNN cultures, which were examined four times at weekly intervals, before being discarded as negative.

0003–4983/79/050433+05 $01.00/0

TABLE

Details of rodents naturally infected with L. major *including tissues shown by N.N.N culture to contain parasites*

No.	*Species*	*Sex M/F*	*Estimated date of birth*	*Date of Capture*	Leishmania *in* *Spleen*	*Liver*	*Bone-marrow*	*Blood*
34	*Mastomys erythroleucus*	F	Dec. 1976/Jan. 1977	6 July, 1977	+	−	−	−
51	*Mastomys erythroleucus*	M	Jan.–Feb., 1977	8 July, 1977	+	+	−	−
52	*Tatera gambiana*	F	Feb.–March, 1977	1 September, 1977	−	−	+	−
61	*Mastomys erythroleucus*	M	Feb.–March, 1977	2 September, 1977	+	+	−	−
95	*Tatera gambiana*	F	October, 1977	9 March, 1978	−	−	−	−

All four rodent strains, (three from *Mastomys* and one from *Tatera*), showed the same enzyme variant types: MDH, type XIII; GPI, type III; G6PDH, type IV; and 6PGDH, type IV. Two excreted factor sub-serotypes were distinguished: B_2 (one *Mastomys* strain) and A_1B_2 (two *Mastomys* and *Tatera* strains). The nuclear and kinetoplast buoyant densities of DNA of one of the strains from *Mastomys* were 1·719 g/ml and 1·703 g/ml respectively.

DISCUSSION

Ranque (1973) found five infected *Arvicanthis niloticus* among 331 trapped in the Thies region; 148 *Mastomys* and 48 *Tatera* trapped during the same period in the same area were not infected.

Our results from another area of the same region show that *Arvicanthis niloticus* is not the only reservoir of cutaneous leishmaniasis in Senegal. Only three *Arvicanthis* were trapped during the present work. The *Arvicanthis* populations were low in the whole of Senegal in 1976 and 1977 due presumably to a periodic sudden fall in the population. *Tatera gambiana* and *M. erythroleucus* are also infected and show high rates of infection: two out of 36 *Tatera* and three out of 41 *Mastomys*.

Infections of *Mastomys* and *Tatera* appear identical to those of *Arvicanthis*: apparently healthy animals without cutaneous lesions but with parasites in internal organs.

The time of infection from the approximate date of birth and the dates of capture of animals (Table) was between February and July in two cases, March and August in another two cases and October and March in the last rodent. Thus, transmission is independent of the seasons and it is known that adult *Phlebotomus duboscqi* were found in the entrances of burrows throughout the year. The rodent strains were identical to those isolated from human cases and *P. duboscqi* in the same area on the basis of enzyme variant types, DNA buoyant density and amastigote size. These strains are established as *L. major* since they possess the same enzyme variants and DNA buoyant densities as isolates of *L. major* from the USSR and Israel. The measurement of the longest diameter of these strains conform fairly closely to those obtained for *L. major* (=*L. tropica major*) by Yakimoff (1915), i.e. a maximum of 5·49 μm and Kellina (1962) i.e. an average of 4·48 μm.

Two excreted factor subserotypes were associated with these strains. One human strain, a strain from *P. duboscqi*, a strain from *Tatera* and two strains from *Mastomys* were subserotype A_1B_2, which is also associated with strains from Libya (Ashford *et al.*, 1976; Ashford *et al.*, 1977) and Israel (Schnur and Zuckerman, 1976). Three human strains and one from

Mastomys were subserotype B_2, a subserotype associated with a large number of African isolates of differing origins (Chance *et al.*, 1978). The four human strains were isolated from cases from the same village.

ACKNOWLEDGEMENTS. We are grateful to Dr. R. S. Bray for his help and advice and to Dr. J. Renaudet for the histopathological examinations. We are also indebted to the monks of the Keur Moussa Monastery for allowing us to work in their grounds. Thanks are also expressed to Mrs. Ann-Marie Simon, Mr. Kader Mariko and Mamadou Tall for their technical assistance.

REFERENCES

ASHFORD, R. W., CHANCE, M. L., EBERT, F., SCHNUR, L. F., BUSHWERER, A. K. & DREDI, S. M. (1976). Cutaneous leishmaniasis in the Libyan Arab Republic: distribution of the disease and identity of the parasite. *Annals of Tropical Medicine and Parasitology*, **70**, 401–410.

ASHFORD, R. W., SCHNUR, L. F., CHANCE, M. L., SAMAAN, S. A. & AHMED, H. N. (1977). Cutaneous leishmaniasis in the Libyan Arab Republic: preliminary ecological findings. *Annals of Tropical Medicine and Parasitology*, **71**, 265–271.

CHANCE, M. L., PETERS, W. & SHCHORY, L. (1974). Biochemical taxonomy of *Leishmania*. I. Observations on DNA. *Annals of Tropical Medicine and Parasitology*, **68**, 307–316.

CHANCE, M. L., SCHNUR, L. F., THOMAS, S. C. & PETERS, W. (1978). The biochemical and serological characterisation of leishmanial strains from the Aethiopian zoogeographical region. *Annals of Tropical Medicine and Parasitology*, **72**, 533–542.

DEDET, J.-P., DEROUIN, F. & CORNET, M. (1978). Infestation spontanée de *Phlebotomus duboscqi* par des promastigotes de *Leishmania* au Senegal. *Compte rendu de l'Académie des sciences, Paris, Séries D*, **286**, 301–302.

GARDENER, P. J., CHANCE, M. L. & PETERS, W. (1974). Biochemical taxonomy of *Leishmania*. II. Electrophoretic variation of malate dehydrogenase. *Annals of Tropical Medicine and Parasitology*, **68**, 317–325.

HUBERT, B. & ADAM, F. (1975). Reproduction et croissance en élevage de quatre espèces de rongeurs sénégalais. *Mammalia*, **39**, 57–73.

KELLINA, O. I. (1962). On the dimensions of *Leishmania tropica major* and *Leishmania tropica minor*. *Medical Parasitology and Parasitic Diseases* (in Russian), **31**, 716–718.

LARIVIÈRE, M. (1966). Aspects cliniques et épidémiologiques de la leishmaniose cutanée au Sénégal. *Bulletin de la Société de Pathologie Exotique*, **59**, 83–98.

LARIVIÈRE, M., CAMERLYNCK, P., RANQUE, P. & VILLOD, M. T. (1965). *Arvicanthis* sp. réservoir de virus naturel possible de *Leishmania tropica* au Sénégal. *Compte rendu de l'Académie des sciences, Paris, Séries D*, **260**, 4869–4870.

RANQUE, P. (1973). *Études morphologique et biologique de quelques Trypanosomides récoltés au Senegal*. Thèse Sciences Marseille. 378 pp.

RANQUE, P., QUILICI, M. & CAMERLYNCK, P. (1974). *Arvicanthis niloticus* (Rongeur, Muridé) réservoir de virus de base de la leishmaniose au Sénégal. *Bulletin de la Société de Pathologie Exotique*, **67**, 167–175.

RIOU, M. & ADVIER, M. (1933). Leishmaniose cutanée contractée au Sénégal. *Bulletin de la Société de Pathologie Exotique*, **26**, 254–256.

SCHNUR, L. F. & ZUCKERMAN, A. (1976). Excreted factor (EF) serotypes of Israeli leishmanial strains. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 15.

SCHNUR, L. F. & ZUCKERMAN, A. (1977). Leishmanial excreted factor (EF) serotypes in Sudan, Kenya and Ethiopia. *Annals of Tropical Medicine and Parasitology*, **71**, 273–294.

SCHNUR, L. F., ZUCKERMAN, A. & GREENBLATT, C. L. (1972). Leishmanial serotypes as distinguished by the gel diffusion of factors excreted *in vitro* and *in vivo*. *Israel Journal of Medical Sciences*, **8**, 932–942.

YAKIMOFF, W. L. (1915). Contribution a l'étude des leishmanioses de l'homme et du chien dans le Turkestan russe. *Bulletin de la Société de Pathologie Exotique*, **8**, 474–503.

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset

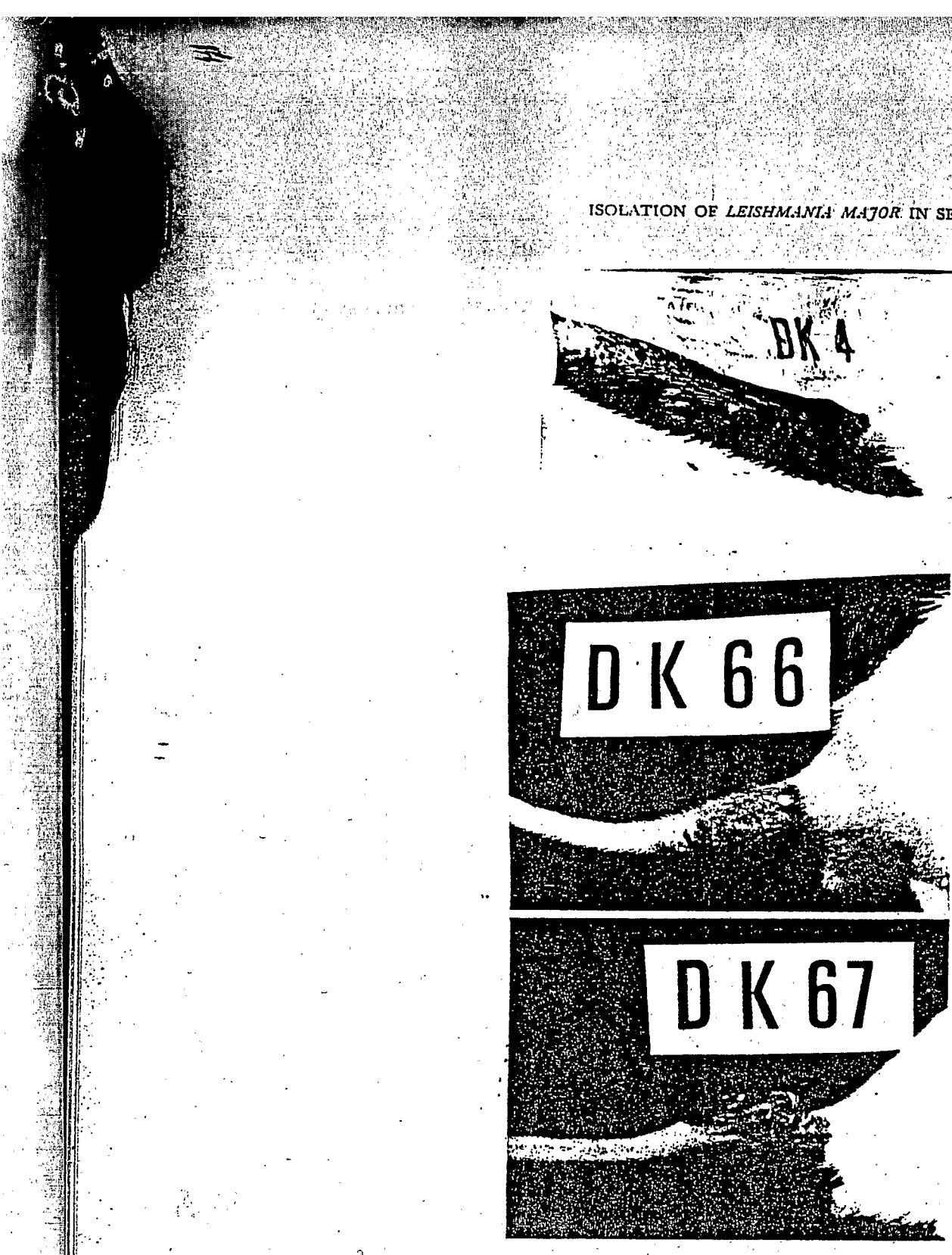

Fig. 1. Tail lesions of white mice inoculated with a human strain (DK 4), a strain from *Mastomys* (DK 66) and a strain from *Tatera* (DK 67).

Strains

The pathogenicity of one strain from *Mastomys* and another from *Tatera* was examined by injecting 0·2 ml of a culture intradermally into the tails of white mice. Impression smears and histological sections were prepared from the lesions produced. The impression smears were used for morphometric determinations. The strains were characterized by the following biochemical and serological techniques. The electrophoretic variants of malate dehydrogenase (MDH), glucose phosphate isomerase (GPI), glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) and the buoyant density of nuclear and kinetoplast DNA were determined by the methods of Chance *et al.* (1978). The serotype of excreted factors (EF) was determined by the method of Schnur *et al.* (1972) and Schnur and Zuckerman (1977).

RESULTS

A total of 102 rodents were trapped between April 1977 and April 1978: five *Heliosciurus gambianus*; 36 *Tatera gambiana*; 11 *Taterillus pygargus*: four *Cricetomys gambianus*; one *Rattus rattus*; three *Arvicanthis niloticus*; one *Myomys daltoni*: 41 *Mastomys erythroleucus*.

Five (three *M. erythroleucus* and two *T. gambiana*) were found naturally infected with leishmanial parasites (Table). No cutaneous lesions were seen in these rodents.

The experimental inoculation of promastigotes into white mice resulted in the development of lesions containing amastigotes. These were similar to the lesions which followed the inoculation of promastigotes of strains isolated from man and *P. duboscqi* (Fig. 1). The longest diameters of amastigotes in smears from these lesions were similar to those in smears from human lesions (means 4·15–5·21 μm) (Fig. 2). All the lesions showed the same histopathology, i.e. that of a typical granuloma containing numerous amastigotes in mononuclear histiocytes.

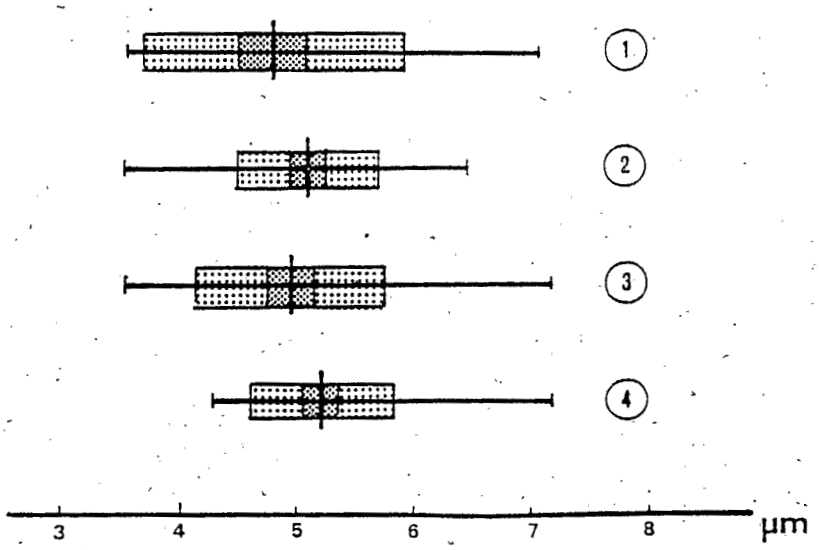

Fig. 2. Dice-Leraas diagram of the longest diameter of amastigotes of various Senegalese strains: the human strain DK 102 (1), the human strain DK 104 (2), the strain from *Mastomys* DK 66 (3), and the strain from *Tatera* DK67 (4).