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ECOLOGY OF LEISHMANIASIS IN THE SOUTH OF FRANCE
19. Determination of the hosts of *Phlebotomus ariasi* Tonnoir, 1921 in the Cévennes by bloodmeal analyses[1]

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SUMMARY. Engorged sandflies were collected using 58 CDC light traps set up nightly for 5 weeks at 12 stations in the commune of Roquedur, Gard, France, in the Cévennes focus of leishmaniasis. Of 782 engorged females, 593 were *Phlebotomus ariasi*, 3 were *P. mascittii* and 186 were not identified. Using the precipitin ring test and counter-current immuno-electrophoresis, the bloodmeals were tested with antisera to man, leporids, rodents, canids, mustelids, equids, suids, bovids, birds, lizards and amphibia. Of 530 identified bloodmeals, 2 were avian blood, 26 were mixed meals of mammalian blood and 25 were meals taken from unidentified mammals. Of the 477 other sandflies, 211 (44.2%) had fed on canids, 107 (22.4%) on man, 70 (14.7%) on bovids (presumed to be cattle at one station and goats elsewhere), 33 (6.9%) on leporids (probably mostly domesticated rabbits), 26 (5.5%) on mustelids (probably badgers), 17 (3.6%) on horses and 13 (2.7%) on rodents (probably brown rat). The proportions of feeds on different mammals varied according to their availability at each station. When both man and dog were equally available, the dog was preferred. The finding that, away from human habitation, *P. ariasi* commonly feeds on mustelids suggests the need for a reappraisal of animals of this family as possible reservoirs of leishmaniasis in the Cévennes.

Écologie des leishmanioses dans le sud de la France. 19. Identification par l'analyse des repas sanguins, des hôtes de *Phlebotomus ariasi* Tonnoir, 1921 en Cévennes

RÉSUMÉ. Une série de captures de Phlébotomes gorgés a été réalisée pendant cinq semaines, à l'aide de 58 pièges lumineux CDC, dans 12 stations réparties sur la commune de Roquedur (Gard, France) au sein du foyer cévenol de leishmaniose viscérale. Sur 782 femelles gorgées capturées, 593 *P. ariasi* et 3 *P. mascittii* ont été déterminées. Les tests de l'anneau de précipitine et de l'électrosynérèse ont permis d'identifier les repas sanguins. Plusieurs antisérums ont été utilisés, correspondant aux Léporidés, Rongeurs, Canidés, Mustelidés, Equidés, Suidés, Bovidés,

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Oiseaux, Lézards, Amphibiens et à l'Homme. L'analyse de 530 repas montre que deux d'entre eux ont été prélevés sur les Oiseaux, 26 correspondent à des mélanges de sang de Mammifères. Dans 25 cas, il s'agit de Mammifères non identifiables. L'examen des 477 autres échantillons montre que 211 Phlébotomes se sont gorgés sur le Chien (44,2%), 107 sur l'Homme (22,4%), 70 sur des Bovidés (vaches et chèvres, 70, 1%), 33 sur des Léporidés, sans doute domestiques (6,9%), 26 sur des Mustélidés, probablement le Blaireau (5,5%) 17 sur les Chevaux (3,6%) et 13 sur des Rongeurs, vraisemblablement le Surmulot (2,7%). La proportion des divers hôtes varie pour chaque station selon les possibilités offertes : lorsque l'Homme et le Chien sont également présents, on observe une nette préférence pour ce dernier. Le fait que *P. ariasi* puisse se gorger sur des Mustélidés, en dehors des zones d'habitat humain, amène à reconsidérer le rôle de ces animaux en tant que réservoir de la leishmaniose en Cévennes.

Phlebotomus ariasi is the proven vector of *Leishmania infantum* in the Cévennes, Southern France (cf. R. Killick-Kendrick and J.-A. Rioux, 1981). In experimental conditions, varying proportions of female specimens of this fly will engorge on man, dog, chicken, fox or bat (J.-A. Rioux *et al.*, 1969). To discover the range of vertebrates on which it will feed in nature and, in particular, to find out on which animals it engorges at sites away from human habitation, we identified bloodmeals of wild caught flies by the precipitin ring test and by counter current immuno-electrophoresis (CCIE). The results are presented here.

Materials and methods

Study area and trapping sites

The study area in the commune of Roquedur, Gard, is described by J.-A. Rioux *et al.* (1979) and R. Killick-Kendrick *et al.* (1984). At each of ten locations chosen as trapping sites five CDC traps powered either by 12 volt batteries or mains electricity were set up in line about 1.5 m above ground. Similarly, in an adajcent valley, four CDC traps were set up at each of two other collecting stations. Descriptions of the trapping sites, with notes on hosts known to be available, are as follows :

Laumède. Map réf. l 1.481g L 48.866g. Alt. 450 m
Inhabited hamlet ; many dogs ; badger set within easy flight range ; chicks and domestic (? and wild) rabbits present ; no livestock.

Roubignac. Map réf. l 1.454 L 48.866. Alt. 520 m
Large inhabited farm in adjacent valley with cattle, horses, chickens and dogs.

Mas d'Arboux. Map réf. l 1.487g L 48.863. Alt. 350m

La Salle. Map réf. l 1.487g L 48.861g. Alt. 320 m
Two adjacent inhabited hamlets within easy flight range of each other ; dogs, goats, domestic rabbits and brown rats present ; horses and cattle absent ; badger set within flight range.

"Cromwell". Map réf. l 1.471g L 48.856g. Alt. 600 m
Small isolated ruin within flight range of an inhabited smallholding with dogs, goats and wild rabbits present ; horses and cattle absent. Highest point in valley.

Langlade. Map réf. l 1.475g L 48.859g. Alt. 540m
Isolated house now occasionally used as a holiday home ; no livestock ; within flight range of the same smallholding as "Cromwell".

"Church". Map réf. l 1.481g L 48.868g. Alt. 510m
Abandoned church ; no habitations ; within easy flight range of Laumède.

Le Vernet. Map réf. l 1.453 g L 48.858. Alt. 430 m
In adjacent valley ; old isolated farm left vacant until team's occupation ; no dogs or bovids ; horses near house.

"Ubac I". Map. réf. l 1.483g L 48.856g. Alt. 470 m

"Ubac II". Map réf. l 1.488g L 48.856g. Alt. 440 m
About 500 m apart on side of a track far from human habitation ; visited nightly often with dog ; no livestock ; within flight range of a badger set.

La Chapelle. Map réf. l 1.488g L 48.859g. Alt. 320 m
Chapel close to inhabited hamlet at lower end of the valley.

La Rabasse. Map réf. l 1.489g L 48.861g. Alt. 170 m
Holiday house occasionally occupied by people and dog ; at the bottom of the valley ; generally unfavourable habitat for sandflies.

Collection and storage of engorged sandflies

The traps were run from just before sunset to dawn when the cages were collected and taken to the field laboratory at Laumède. Engorged sandflies were separated from other insects, killed with CO_2, identified by examination of the spermathecae and then stored individually in numbered stoppered tubes containing silica gel. The tubes were kept in desiccators at room temperature until the bloodmeals were tested.

Preparation of antisera

The bloodmeals were tested with antisera prepared against man, leporids, rodents, canids, mustelids, equids, suids, bovids, gallinaceous birds and with general avian and general mammalian antisera.

All mammalian antisera except that against leporids were prepared by the method of P. F. Boreham and G. S. Gill (1973). With the exceptions of the anti-mustelid and anti-leporid antisera, the titres ranged from 1 : 256,000 to 1 : 512,000 and there were no cross reactions with heterologous sera at dilutions of 1 : 10.

The anti-mustelid antiserum was prepared in rabbits with the serum of the badger *(Meles meles)* and cross reacted with serum of the ferret *(Putorius putorius furo)* but not with that of ox, sheep, pig, man, horse, dog or cat. An attempt to

absorb out the cross reaction to ferret was unsuccessful and the antiserum was used at a final titre of 1 : 128,000. The cross reaction showed that the antiserum could not be used to distinguish between subfamilies Melinae [of which the Eurasian badger *(Meles meles)* was seen in the study area] and Mustelinae : the distribution of the weasel *(Mustela nivalis)*, polecat, *(P. putorius)*, pine marten *(Martes martes)* and beech marten *(Martes foina)* includes the study area (van den Brink, 1972).

The anti-leporid serum was commercially prepared with rabbit serum in goats (Miles-Scientific. Ltd.). Its homologous titre was 1 : 64,000 and it did not cross react with the sera of ox, sheep, pig, horse, dog or cat. A cross-reaction with human serum at a titre of 1 : 100 was absorbed before use.

The general mammalian antiserum was prepared by the method of P. F. Boreham and G. S. Gill (1973) with pooled sera of mammals of eighteen different families.

Of the two avian antisera, the one against gallinaceous birds was prepared from the albumin fraction of pooled sera consisting of equal volumes of serum of chicken, turkey, pheasant and guinea fowl (G. S. Gill, 1983). The homologous titre was 1 : 512,000 and it did not cross-react with sera of birds of six other Orders. The general avian antiserum was prepared with the pooled sera of birds of five different Orders (Galliformes, Columbiformes, Passeriformes, Anseriformes and Ciconiiformes) by the method of P. F. Boreham and G. S. Gill (1973). Its homologous titre was 1 : 128,000.

Methods of bloodmeal identification

All bloodmeals were identified using both of the following techniques :

Precipitin ring test. On removal from the desiccator, each fly was crushed in 0.2 ml of 0.85% saline and the eluted bloodmeals were kept overnight at 4° C. The precipitin ring test was performed by the method described by P. F. Boreham (1972). Results were read after 30 minutes, 60 minutes and 120 minutes.

Counter current immuno-electrophoresis (CCIE). Glass plates (7.5 × 5 cm) were coated with 1% oxoid gel (Code L 28, Oxoid Ltd., England) in barbiturate gel buffer pH 8.6. Wells 1.5 mm in diameter and 2 mm apart were punched in the gel. 5 µl of the bloodmeal eluate was pipetted into the cathodic well and 5µ l of the antiserum into the anodic well. The electrophoretic tank was filled three-quarters full with sodium barbitone buffer pH 8.6 (Hirschfield, 1960) and the plates, charged with eluates and antisera, were placed face down over the tank bridge. Contact between the tank buffer and the plates was by paper wicks 7.5 cm wide (Whatman 3 MM chromatography paper). For each electrophoretic run, the current was maintained at 100 volts and 5 mA per plate for 15 minutes. After the run, the plates were washed for 4 hours in two changes of saline then for a further 2 hours in distilled water. They were then covered with damp filter paper and allowed to dry overnight at 37° C. On the following day, the plates were stained with amido-black for 5 minutes using the method of J. Hirschfield (1960). A fine line of precipitate between the two wells indicated a positive result.

Results

A total of 782 engorged sandflies were caught at 10 of the 12 collecting stations on 30 nights in July and August 1980. None was caught at the 2 lowest stations in the valley, La Rabasse and La Chapelle. Of the 782 flies, 593 were identified by the examination of freshly dissected spermathecae as *P. ariasi*, 3 as *P. mascittii* and the remaining 186 were not identified. Bloodmeals of 63 of the 593 *P. ariasi* were unidentified either because the blood was too well digested or because gravid females had been mistaken for engorged flies. Of the remaining 530, none had fed on reptiles, 2 had fed on birds (one on a galliform and the other on a non galliform) and 528 had fed on mammals. Of this last group, 25 had fed on mammals which could not be identified. Hosts on which the remaining 503 sandflies had fed were identified to suborder (Rodentia), family (Leporidae, Canidae, Mustelidae, Equidae, Bovidae) or species (man, the only Primate present). 477 of the flies had fed on blood of a single species of host — presumably the same individual — and 26 had feeds of mixed blood *viz* : man/equid (6), man/canid (2), man/leporid (3), man/mustelid (2), man/bird (1), man/bovid (1), canid/bovid (1), canid/leporid (1), bovid/leporid (7) and man/mustelid/equid (2). The results of the tests of the bloodmeals of the 477 sandflies with unmixed meals are shown in *Table I* : The three engorged *P. mascittii* caught at the project base (Laumède) were also tested ; one had fed on a canid, one on a leporid and the bloodmeal of the third was unidentifiable.

Discussion

Although the precipitin ring test has been extensively used for the identification of bloodmeals of mosquitoes and tsetse flies, it is of limited use with sandflies. Only 5 or 6 tests can be carried out due to the small size of the meal which is generally 0.5 mg or less in weight (O. Theodor, 1936 ; P. D. Ready, 1979). More tests can be carried out with CCIE as this requires only 3-5 µl of eluted bloodmeal per test. Initially CCIE proved to be the less sensitive of the two methods and each result was confirmed by carrying out the more sensitive precipitin ring test. Subsequently, the CCIE has been improved and is now our method of choice for tests on bloodmeals of sandflies.

P. ariasi is gonotrophically concordant (E. Guilvard *et al.*, 1980, T. J. Wilkes *et al.*, 1985) and thus normally takes only one bloodmeal in each gonotrophic cycle. The 26 mixed meals of ten combinations of species of hosts were, therefore, of flies which had presumably been disturbed and had completed their meals on individuals of other species. Nine of the 26 sandflies with mixed meals were caught at station Roubignac, a farm with the widest range of available hosts of any station.

The results of the tests on flies with unmixed bloodmeals show that, in nature, *P. ariasi* appears never to feed on lizards, rarely to feed on birds but engorges on a

TABLE 1. — Numbers of unmixed bloodmeals of *P. ariasi* grouped according to hosts and collecting stations

Station	Canidae	Man	Bovidae	Leporidae	Mustelidae	Equidae	Rodent	Totals
Laumède	78 (78.8%)	0	0	17 (17.2%)	4 (4.0%)	0	0	99
Roubignac	36 (38.3%)	11 (11.7%)	41 (43.6%)	0	0	6 (6.4%)	0	94
Mas d'Arboux	39 (44.8%)	24 (27.6%)	10 (11.5%)	4 (4.6%)	0	1 (1.1%)	9 (10.3%)	87
La Salle	40 (59.4%)	13 (19.4%)	5 (7.5%)	5 (7.5%)	0	0	4 (6.0%)	67
"Cromwell"	6 (13.6%)	20 (45.5%)	8 (18.2%)	3 (6.8%)	7 (15.9%)	0	0	44
Langlade	4 (12.5%)	18 (56.3%)	4 (12.5%)	0	6 (18.8%)	0	0	32
"Church"	6 (27.3%)	10 (45.5%)	1 (4.5%)	1 (4.5%)	4 (18.2%)	0	0	22
Le Vernet	0	1 (9.1%)	0	0	0	10 (90.9%)	0	11
"Ubac 2"	2 (18.2%)	7 (63.6%)	0	1 (9.1%)	1 (9.1%)	0	0	11
"Ubac 1"	0	3 (30.0%)	1 (10.0%)	2 (20.0%)	4 (40.0%)	0	0	10
Totals	211 (44.2%)	107 (22.4%)	70 (14.7%)	33 (6.9%)	26 (5.5%)	17 (3.6%)	13 (2.7%)	477

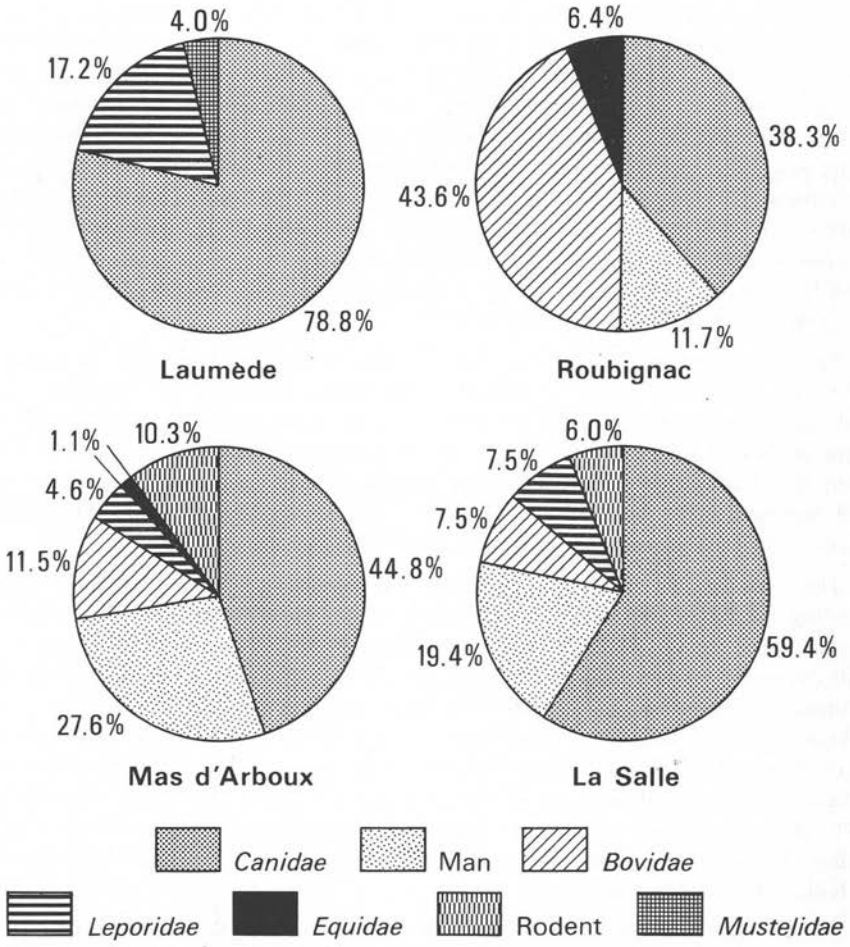

FIG. 1. — The proportions of bloodmeals from different hosts at four of the collecting stations. The total numbers of engorged flies caught at each were ; Laumède : 99, Roubignac : 94, Mas d'Arboux : 87 and La Salle : 67.

wide range of mammals. This range includes man, canids*, mustelids, bovids, equids, leporids and, occasionally, rodents. In a series of experiments in which wild-caught *P. ariasi* were caged with animals of different species, J.-A. Rioux *et al.* (1969) found that the flies did not feed on a lizard *(Lacerta viridis)*, a snake *(Elaphe longissima)*, dormouse *(Eliomys quercinus)*, edible dormouse *(Glis glis)*, the long-tailed field mouse *(Apodemus sylvaticus)* and the black rat *(Rattus rattus)*. The percentages

* Bloodmeals from dogs and foxes, both of which are proven hosts of *L. infantum* in the study area (see Killick-Kendrick & Rioux, 1981) are not separable by the tests used.

which engorged on other animals were 91% on a dog, 76% on a chicken, 55% on a fox, 26% on a rabbit and 10% on a bat (*Rhinolphus* sp.). These results are discordant with the tests on bloodmeals of wild-caught flies in two respects. Firstly, we found only one feed from a gallinaceous bird, even at Roubignac where chickens were plentiful, whereas three quarters of the flies caged with a chicken took bloodmeals. Caging sandflies with animals may, however, induce a willingness to feed on hosts which are not necessarily commonly fed upon in nature. Secondly, no caged sandflies fed on murine rodents but we found that blood of 13 (8.4%) of 154 engorged *P. ariasi* collected at Mas d'Arboux and La Salle reacted with a general rodent antiserum. These feeds were probably on brown rats *(Rattus norvegicus)*, specimens of which were seen at Mas d'Arboux during the work.

From the occasional sightings and the rare finding of droppings, it is known that there are only a few wild rabbits in the study area and the majority of the 33 bloodmeals from leporids were almost certainly from domestic rabbits housed at Laumède, Mas d'Arboux, La Salle or at an inhabited hamlet (Roquedur-le-Haut) within flight range of "Cromwell" and Langlade. It is nevertheless possible that the 4 leporid feeds at "Church", "Ubac 1" and Ubac 2" were from wild rabbits or hares ; the latter animal is, however, extremely rare in the area.

The proportions of bloodmeals from different hosts varied at each station according to the availability of the animals *(fig. 1)*. At Laumède, Mas d'Arboux and La Salle, which are permanently inhabited hamlets with dogs present, the highest proportions of feeds were from canids (78.8%, 44.8% and 59.4% respectively). In contrast, at Roubignac, an inhabited farm where man, dogs, cattle, horses and chickens were available, the largest proportion of feeds was on bovids (43.6%). The comparatively high proportions of feeds from man at three stations which were uninhabited during all or most of the time of the study (45.5% at "Cromwell", 56.3% at Langlade and 45.5% at "Church") are explained by feeds from workers visiting the stations nightly in a concomitant study of the dispersal of sandflies (R. Killick-Kendrick *et al.*, 1984).

An unexpected finding was that, in four uninhabited places in the valley ("Cromwell", Langlade, "Church", "Ubac 1" and "Ubac 2"), 22 (18.5%) of 119 feeds were taken from mustelids, probably badgers, sets of which were in flight range of these stations. Although no mustelids have yet been found infected with *Leishmania* in France (J.-A. Rioux *et al.*, 1969 ; J. Ranque *et al.*, 1978), there are reports from the USSR of *Leishmania* in a badger *(M. meles)* in Georgia (G. M. Maruashvili and B. G. Bardzhadze, 1977), in a weasel *(M. nivalis)* in Kazakhstan (Y. A. Dubrovsky, 1966) and in a marbled polecat *(Vormela peregusna)*, also in Kazakhstan (Faizulin, cited by Y. A. Dubrovsky, 1975). None of the mustelid parasites has been fully characterized although the isolate (MEL/SU/76/ME) from skin lesions on the legs of the badger was shown to be an independent serotype close to *L. major* (B. G. Bardjadze and V. M. Saf'janova, 1981). The finding that *P. ariasi* commonly feeds on mustelids, coupled with the reports from the Soviet Union, suggests a reappraisal of the possible role of these animals as hosts of *L. infantum* in the Cévennes.

The differences in the results at different stations emphasize the need for sampling in a variety of places in a given habitat if the conclusions are to be of any real value. The wide variety of hosts on which *P. ariasi* will feed in nature, according to availability, obscures obvious preferences. There is, nevertheless, evidence of a preference of dog over man from the results at Laumède where both hosts were constantly available : of 99 unmixed bloodmeals identified at this station, 78 (78.8%) were blood of canids and none was from man. This observation partly explains the high prevalence of canine leishmaniasis in the Cévennes compared to the currently low incidence of visceral leishmaniasis in man (G. Lanotte *et al.*, 1979 et 1980 ; R. Killick-Kendrick and J.-A. Rioux, 1981).

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