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## • Introduction

- The *Phlebotominae* include many genera of blood-feeding (hematophagous) flies, including the primary vectors of leishmaniasis. Species belonging to the genus *Phlebotomus* are responsible of the transmission of this parasitic disease in Europe. Leishmaniasis is a zoonotic disease, widely spread all over the world, and an endemic disease in some Spanish regions.
- Within the Autonomous Region of Madrid, starting in 2009, some southern populations were affected by an unexpected outbreak. Since then, 791 human cases have been reported in the region, the vast majority related to the outbreak. After the municipality of Fuenlabrada, Leganés has had the largest number of affected inhabitants with 99 cases (12,5%).
- As a result, since 2012, a Systematic Sand Fly Surveillance Program has been implemented in the area. Based on both castor oil traps and CDC light traps. The results of this intensive entomological monitoring has proven critical to manage the outbreak. Identification up to species level of all samples has produced valuable information on phenology and ethology of sand flies in the southern area of the region of Madrid.

## • Material and Methods

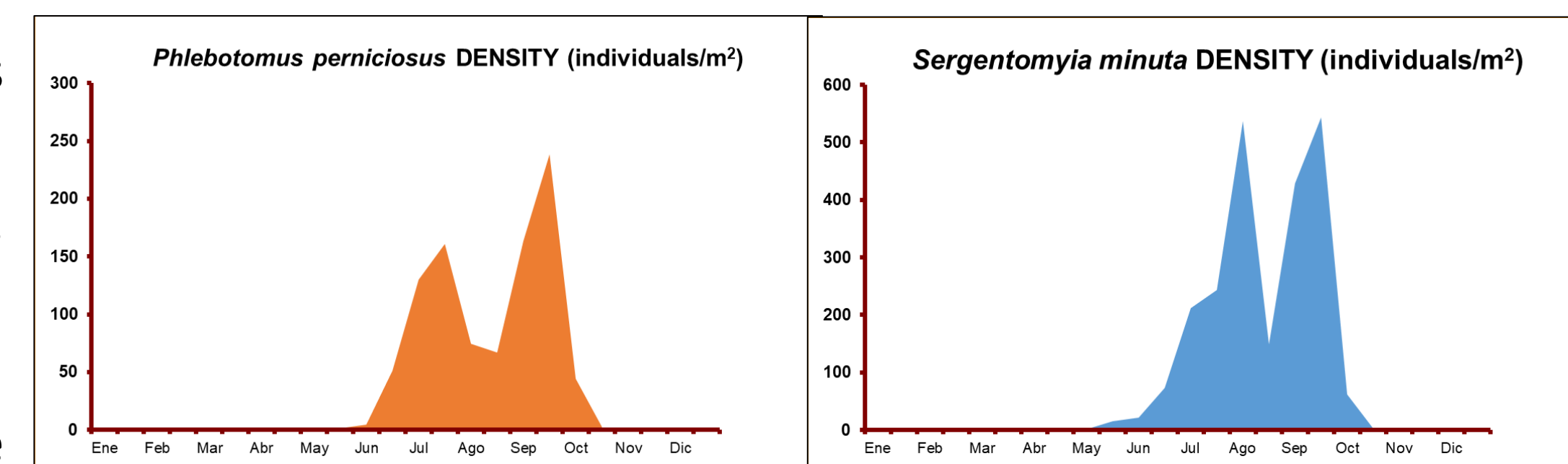
- Surveillance period starts in May and finishes in November. Traps for adults (previous phases are cryptic and very difficult to find) are checked every two weeks and the number of specimens are counted and later identified, if possible, in the laboratory.
- Most of the monitoring is based on castor oil traps (Fig. 1). That consist of a white sheet of paper, A5 size, impregnated in the oil and pierced by a stick, that is later used to fix the trap to the surface. These traps are placed in appropriate places (holes, cavities, roots, burrows, etc.). They give us information on the presence of sand flies, their distribution and density.
- Additionally, CDC light traps were used to trap live females, analyze them in the laboratory, identify the animals they are feeding on and the presence of *L. infantum*. 1 trap was in Leganés and 3 were in Fuenlabrada.
- Oil traps, once collected, are kept in a cold room at 4 °C until they are handled to separate, process and identify the captured sandflies.
- Trap position was grouped by stations. That were located close to cases of human leishmaniasis, in particular those that would have affected children under 2 years of age and people over 70 years of age. Leganés has a total of 5 stations, from 2012 to 2021 a total of 2.181 oil traps have been processed.



Fig. 1. Castor oil trap before and after field use; Trap detail; Male and Female of *P. perniciosus* after laboratory processing; and example of an adult specimen photographed in the area.

## • Results

- A total of 73.818 sand flies from the 2.738 oil traps have been identified from 2012 to 2021.
- 64% of the individuals were *Phlebotomus perniciosus*, the other main species was *Sergentomyia minuta* and only 4 individuals of *Plebotomus papatasi* were trapped.
- Less than 13% of the *P. perniciosus* captured were females. In contrast, this percentage increases to just over 43 for *S. minuta*.
- The average number of sand flies per trap has decreased irregularly from almost 50 in 2012 to 21 in 2021 (29 individuals per trap for the total period).
- From 2012 to 2021, there has been a decrease in the average density from 143,8 fleb/m<sup>2</sup> to about 80 fleb/m<sup>2</sup>.
- Activity starts normally in June and continues until October, with two peaks in July and September, occasioned by the lack of humidity during the driest weeks of summer.
- The trapping points with the highest activity of sand flies are located in the south and southwest section of the municipality. This areas limit with Fuenlabrada, the municipality with the highest incidence of human cases during the outbreak.



Year	<i>Phlebotomus perniciosus</i>			<i>Sergentomyia minuta</i>			<i>P. papatai</i>	Traps	Points	<i>P. pern.</i> density	<i>S. minu.</i> density	Female P.P.D.
	Male	Female	Total	Male	Female	Total						
2012	8388	1429	9817	1673	1266	2939	1	260	28	605,4	181,2	88,1
2013	6488	1556	8044	2073	1678	3751	1	492	28	262,1	122,2	50,7
2014	7440	1062	8502	1399	800	2199	1	342	27	398,6	103,1	49,8
2015	4621	833	5454	1830	1056	2886	0	268	22	326,3	172,7	49,8
2016	3480	491	3971	2039	1506	3545	0	266	22	239,4	213,7	29,6
2017	3887	296	4183	2829	2134	4963	0	291	22	230,5	273,4	16,3
2018	2324	150	2474	1130	858	2018	1	262	22	151,4	123,5	9,2
2019	1280	34	1314	726	921	1647	0	186	10	113,3	142	2,9
2020	1276	79	1355	425	459	884	0	186	10	116,8	76,2	6,8
2021	2026	56	2082	1003	817	1820	0	185	10	180,4	157,7	4,9
TOTAL	41210	5986	47196	15127	11495	26622	4	2738		312	163,9	44

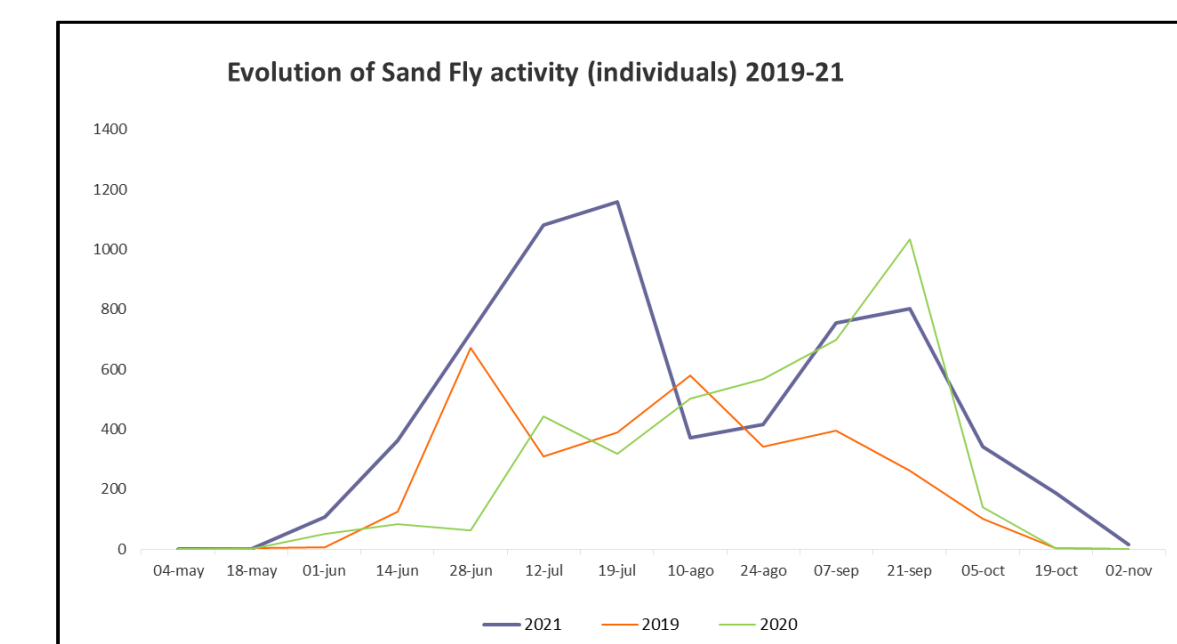
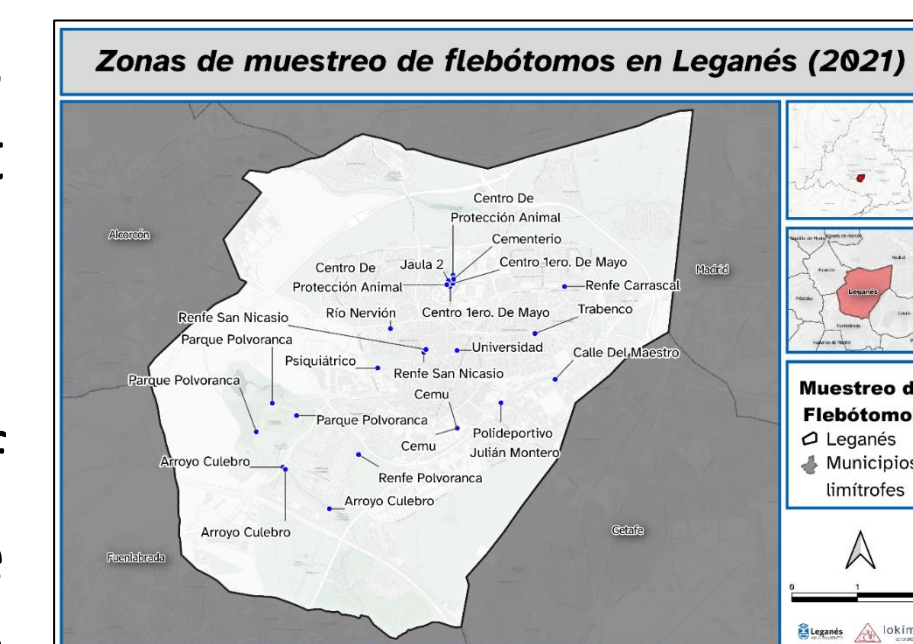


Fig 2. Phenology of *P. perniciosus* and *S. minuta*; results table; surveillance map; and activity graph.

- CDC light traps captured a total of 7.444 female sand flies from 2012 to 2021, including all 4 placed traps. 171 (2,5%) females proved positive to *Leishmania infantum* (microscopy+). For the last years values have been bellow 1% (0% in 2020).
- From 2011 to 2020, 390 samples were analyzed to identify the host females had fed on. 271 (69,5%) corresponded to rabbit, 91 (23,3%) to hare, 18 (4,6%) to cats, 1 (0,3%) to dogs, 1 other animals (0,3%) and 8 (2%) to humans. Consequently, for the same period, prevalence of *Leishmania* (PCR+) was analyzed in 2.167 rabbits and 651 hares (*Lepus granatensis*). 17,6% (381) of rabbits (*Oryctolagus cuniculus*) were positive and 30,7% (200) of hares.
- Since systematic surveillance started, density of sand flies in many of the monitored stations of Leganés have exceeded 100 individuals per squared meter, meaning a high concentration. But a general decreasing tendency has been stable since 2012.
- During 2017, 87 cases of leishmaniasis were reported in the region of Madrid, 15 more cases than in 2016, and 28 (32.2%) were associated with this outbreak. The aggregated incidence rate was 1,34 cases per 100.000 inhabitants. The municipality with the highest incidence rate was Fuenlabrada (6.39 cases per 100,000 inhabitants), followed by Leganés (4.79 cases per 100,000 inhabitants).
- In recent years there has been a significant decrease in the number of cases. Since 2020 there have been 15 cases in the outbreak area, of which 3 correspond to the municipality of Leganés. Previously, there was 6 cases in 2009, 97 cases in 2010, 197 cases in 2011, 160 cases in 2012, 92 cases in 2013, 95 cases in 2014, 47 cases in 2015, 27 cases in 2016 and 28 cases in 2017. From 2009 to 2017, 38.3% of the cases presented visceral Leishmaniasis, 61.7% cutaneous Leishmaniasis and 31.9% required hospital admission.
- Public Health Authorities concluded, as data supports, that the unexpected outbreak of Leishmaniasis in the southern outskirts of Madrid was triggered by changes in the natural environment. A demographic explosion of rabbits and hares due to the rapid urbanization, the abandonment of agricultural uses of land and the lack of natural or human predation, concurring with high densities of *F. perniciosus*, that fed on them and made these leporids an unusual reservoir of *L. infantum*. This situation exposed humans, living in urban areas, to the sand flies and nearby leporids with high prevalence of Leishmaniasis.

## • Conclusions & Further Research

- Vector surveillance is a critical task when dealing with Leishmaniasis cases. Systematic monitoring of sand flies in the southern outskirts of Madrid has proven to be a key aspect to understand the situation and implement precise measures to control the expansion of human Leishmaniasis cases. Actions included: environmental health measures (maintenance and clearing of vegetation, removal of rodent burrows, closure of rainwater pipes, sealing of registers, elimination of cracks in walls and holes in buildings, cleaning of sewer system, etc.), reservoir control (reduction of the population of rabbits and hares, promotion of prevention of Leishmaniasis in dogs and cats, etc.), vector surveillance and control and citizenship communication campaigns.
- Research on sand fly behavior and biology is mandatory. Urbanization and climate change seem to have increased the probability of Leishmaniasis cases, thus, more knowledge on this vector is essential. Also, direct vector control treatments, based on chemical adulticide spraying or fogging, have had very limited results, as previously described. Further studies on alternative direct control measures are required.
- Until human incidence of Leishmaniasis disappears surveillance of sand flies should continue.