What is responsible for a large and unusual outbreak of leishmaniasis in Madrid?

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ABSTRACT

Several areas in the southeast of Madrid (Spain) have been suffering an outbreak of leishmaniasis since 2009. By December 2012 a surprisingly large 446 human infections had been reported, largely among immunocompetent adults. Hares are the suspected reservoir. These observations raise many questions since, in Spain, the victims of leishmaniasis are normally children and the immunodepressed, and dogs are the usual reservoir.

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In Spain, leishmaniasis is an endemic zoonosis caused by *Leishmania infantum*, which is found nearly everywhere in the Peninsula and Balearic Islands. Dogs are the main reservoir of this pathogen, and the sand flies *Phlebotomus perniciosus* and *Phlebotomus ariasi* the main vectors of transmission. In Spain, leishmaniasis has traditionally been regarded as a hypoendemic infection mostly affecting children. In the 1980s, it emerged as an important problem among people carrying the human immunodeficiency virus (HIV) - at least until HAART therapy became available in the 1990s. This significantly reduced the number of patients with AIDS also infected with *L. infantum*, although they are still at greater risk.

Currently, the mean annual incidence of notified leishmaniasis in Spain is 0.45 cases/100.000 inhabitants. Between 2000 and 2010, Spain's National Information System for Hospital Data recorded some 2739 hospitalised patients with a diagnosis of leishmaniasis. As expected, the majority (30.5%) were children under 14 years of age, along with adults coinfected with HIV (36.1%) [1]. In Madrid, along the Mediterranean coast, and in the Balearic Islands, the incidence is somewhat greater (with some 12-25 cases recorded per year, an incidence of 0.5/100,000), but the epidemiological pattern of the disease is the same for the rest of the country [3]. Dogs, however, are much more commonly infected by *L. infantum*. Indeed, some 3.7-34.6% of all dogs are seropositive, depending on the area. About half of those infected develop canine leishmaniasis; it is therefore a serious veterinary problem in Spain [2]. In Madrid, the incidence of canine leishmaniasis is some 7.8% in stray dogs and 5.2% in dogs with owners [4,5]. Canine leishmaniasis seems to be most common in periurban and suburban areas where townhouses with a patio or small garden are common (in the city, apartment blocks are the norm). These gardens are good places for the vector to develop; they also allow it to come into contact with the pathogen's normal canine hosts. This has led to the 'domestication' of the *L. infantum* transmission cycle; indeed, most canine infections now occur in these suburban-type areas.

A few years ago, however, a large and unusual outbreak of leishmaniasis began in Madrid's southeast. Between mid 2009 and the end of 2012, a surprising 446 human infections were recorded – 35.9% visceral and 64.1% cutaneous. The epidemiology of this outbreak is surprising too. First it is urban in nature rather than periurban/suburban, and second, only 14.1% of those affected have been children under 14 years of age. Further, just 15% of those infected have been immunocompromised individuals (11% immunosuppressed, 4% with HIV). Indeed, 70.6% of all those infected have been

immunocompetent adults (both sexes) [6]. Such figures are quite unheard of for human leishmaniasis in Spain.

Since the start of the outbreak, the WHO Collaborating Centre for Leishmaniasis Leishmaniasis at the *Instituto de Salud Carlos III* has been active in trying to understand its epidemiological characteristics, and how it might be brought under control. As a National Reference Laboratory for the disease, it has serologically and molecularly confirmed the diagnosis of most of those affected, has isolated and cultivated the parasite (which is clearly *L. infantum*), and molecularly typed 31 isolates. The latter was performed by studying the polymorphisms in a sequence of internal transcribed spacers (ITS) 1 and 2, as well as the polymorphisms of the gene *haspb* (which codes for hydrophilic acylated surface protein B). The results have shown the isolates involved in this outbreak to belong to the ITS LOMBARDI subtype of *L. infantum* – as did those isolated during other outbreaks in different parts of Madrid since (at least) 1992. Interestingly, this subtype was also identified in three hares during a study on the reservoir involved in the present outbreak (7, 8). The *haspb* gene of 22 of the 31 isolates was 920 base pairs (bp) long, while in the remainder it was between 836 and 962 bp long. *haspb* genes of this size have been described in *L. infantum* isolates from other parts of Madrid not involved in the present outbreak (7).

Entomological studies performed in the area confirmed a very high density of sand flies - 45.3 per m^2 compared to the mean for Madrid of 30 per m^2 [1]. The dominant species in the area was *P. perniciosus*. The mean *Leishmania* infection rate in the female sand flies was 2.4% [6].

Several serological surveys among dogs have been performed, but surprisingly the infection rate recorded among the more than 1000 tested was only 1.6-2.0%. This

explains why veterinarians in the area have reported no increase in cases of canine leishmaniasis. Other animals were therefore monitored (this time using the polymerase chain reaction [PCR]), including cats plus rats, rabbits and hares captured in a park at the edge of the affected area. *L. infantum* infection was detected in a surprising 30% of the hares (*Lepus granatensis*), suggesting these are acting as the pathogen reservoir [6]. The transmission of the parasite from these hares to sand flies was confirmed by xenodiagnosis [8], and it was also confirmed that sand flies take blood meals from hares [9]. These findings, plus the high prevalence of *L. infantum* found in other species of hare across Spain [10], strongly suggest that the hares in the outbreak area are involved in the transmission of the pathogen to humans. Such transmission would normally be very uncommon since human populations and hares do not usually share their living space. However, infrastructure work and alterations to the area's environment undertaken as part of its conversion from agricultural land to urban parkland would appear to have brought a wild transmission cycle into contact with people. This, coupled with a huge increase in the hare population and favourable environmental conditions, explains the large number of people that have become infected [6].

But an important question remains: why have so many *immunocompetent adults* been infected? When transmission cycles have involved dogs, the number of human cases has never been so high, and those who are infected are usually children and the immunocompromised. The most likely reason is scant previous exposure of this human population to the *L. infantum* strains carried by these hares; natural immunity is therefore little developed. The contact of the parasite with a human population naïve for leishmaniasis commonly has grave consequences, as shown by the situation in Sudan [11]. Though Spain is a Mediterranean country, where the pathogen is typically endemic, there could be areas where the population has no immunity, and this should be

taken into account when introducing environmental change that brings about the conditions in which domestic or wild transmission cycles can expand into new areas. Certainly, at the *WHO Collaborating Centre for Leishmaniasis*, we have observed an increase in the number of human cases of leishmaniasis in areas that were previously not endemic for the disease – perhaps due to climate change allowing the vector to expand and the mobility of the disease reservoir. We should take care to monitor the factors that contribute to the emergence and re-emergence of this disease, changes in which already seem to have caused a large leishmaniasis outbreak in Southwest Madrid.

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