Leishmaniasis: an overview in the context of an emerging pathogen in Top End wildlife.
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Introduction

In 2000, investigation into an ulcerative disease in the tails of captive Red Kangaroos (Macropus rufus) resident in the Top End led to the identification of a probable Leishmania species as the cause.¹ The discovery led to some media interest²,³ and the concern of the possibility of spread to humans. This article is a very brief overview of human leishmaniasis with the aim of informing health care professionals and the public about this disease and examining the risks to human health in the NT.

The organism and its life cycle

Leishmania is a genus of a protozoan which is characterised by two life stages; the intracellular amastigote stage which occurs in mammals and the extracellular promastigote stage which occurs in the phlebotomine sandfly vector. There are many species of Leishmania which cause disease in humans (14 main ones often grouped into sub-genera) but they all appear similar on light microscopy and need further enzyme testing or nucleic acid amplification for further species differentiation.⁴

The sandfly is infected by ingesting amastigotes with a blood meal. These are then liberated in the sandfly’s stomach and develop into motile promastigotes, which migrate to the mouthparts and are injected into the mammalian host at the next meal. They are then taken up in the host’s macrophages where they revert to amastigotes and multiply.

Although leishmaniasis is usually transmitted to humans from a mammalian reservoir via a sandfly (zoonotic spread), human-sandfly-human (anthroponotic) spread is recognised in some areas as the major mode of transmission (particularly in the Indian sub-continent). Transmission through blood transfusion is well documented; other routes such as sexual spread, inoculation, congenital transmission and direct spread are possible but are either extremely rare or unreported.⁴

The vector

The only recognised vectors for leishmaniasis are the so-called “true” sandflies in the sub family Phlebotominae of the family Psychodidae.⁵ These are often referred to as phlebotomine sandflies. The 2 important vector genera are Phlebotomus in the Old World and Luizomyia in the New World. These genera are not present in Australia, although Phlebotomus may extend through Southeast Asia to Timor (personal communication, Alan Dyce).

Figure 1. Phlebotomus – a female “Sand Fly”

Phlebotomine sandflies (Figure 1) can transmit viruses (sandfly fever and others), bacteria (Bartonella bacilliformis which causes Oroya Fever in South America) and protozoa (Leishmania).⁴ They are small flies, 1.5-3.5 mm in length and look like very small, hairy mosquitoes. Unlike mosquitoes their wings are held in a V above the body rather than flat across the back.⁴ They are about a quarter the size of mosquitoes and slightly larger than the so-called sandflies (biting midges) which are prevalent in the Top-End. Phlebotomine sandflies can inhabit a large range of habitats from sea level deserts to tropical mountain ranges, although
Table 1. Species, distribution, vector and mamalian hosts of visceral leishmaniasis

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Vector</th>
<th>Mammalian hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. donovani</em></td>
<td>India</td>
<td><em>Phlebotomus</em></td>
<td>Humans</td>
</tr>
<tr>
<td></td>
<td>East Africa (Kenya)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. infantum</em></td>
<td>Mediterranean</td>
<td><em>Phlebotomus</em></td>
<td>Dogs</td>
</tr>
<tr>
<td></td>
<td>North Africa</td>
<td></td>
<td>Foxes</td>
</tr>
<tr>
<td></td>
<td>Middle East</td>
<td></td>
<td>Jackals</td>
</tr>
<tr>
<td></td>
<td>Central Asia</td>
<td></td>
<td>Rodents</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. chagasi</em></td>
<td>Central America</td>
<td><em>Lutzomyia</em></td>
<td>Humans and dogs</td>
</tr>
<tr>
<td></td>
<td>South America</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: This table is an approximate guide only.

Each species has fairly specific ecological requirements. They breed in a variety of habitats involving darkness, humidity and organic matter on which the larvae feed. Breeding places include under leaves in rain forest floor litter, hollow trees, tree buttresses, rocky outcrops, and animal burrows. Some species have adapted to the peridomestic setting and breed in cracks in floors and walls, and stone fences.\(^6\)

**The illness**

There are two major forms of leishmaniasis; visceral and cutaneous. Even though there is a close association between the species of *Leishmania* and the clinical disease, the distinction is somewhat blurred with some species recognised as causing visceral leishmaniasis also causing the cutaneous form in exceptional circumstances or in the presence of severe immunosuppression.\(^4\)

**Visceral leishmaniasis (also known as kala-azar)**

This is a chronic disease characterised by infection of the visceral organs such as the spleen and liver and giving rise to fever, hepatosplenomegaly, lymphadenopathy, pancytopenia and progressive emaciation and weakness.\(^4,7\) The incubation period is generally some months, but can be as long as 10 years.\(^4\) The disease has an effect on both humoral and cell mediated immunity such that secondary bacterial infection is the common cause of death.\(^8\) The prognosis following infection depends on the host’s cell mediated immunity, but once infection is established in the spleen, mortality without treatment is almost invariable.

Visceral leishmaniasis is widely distributed around the Mediterranean basin, tropical Africa, Central and Eastern Asia and parts of South

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*Figure 2. Distribution of visceral leishmaniasis (from Bell\(^9\))*
American Leishmaniasis (see Figure 2). The recognised causal species are *L. donovani*, *L. infantum* and *L. chagasi*. Distribution, vectors and mammalian hosts are given in summary in Table 1.

**Cutaneous leishmaniasis**

This is a chronic ulcerative and deforming disease of the skin and mucous membranes. There is a wide clinical spectrum of disease which is determined among other things by the species of *Leishmania* and the host's cell mediated immunity. Some forms are mild and self-healing, others can remain indolent and recrudesce while others can be diffuse and disseminate. The typical ulcer may be single or multiple and may be on any part of the body, but usually the extremities or the face. The edge is raised and infiltrated but not undermined. Most are painless and lymph nodes can be involved. Depending on the species they can be dry (*L. tropica*) or exudative (*L. major*), although the clinical picture does not always match the species. If cell mediated immunity is poor lesions can be diffuse. One form of the disease (mucocutaneous leishmaniasis) occurs at the mucocutaneous junction, particularly the nose, and after many years can involve ulceration and destruction of the cartilaginous septum and surrounding tissues resulting in severe disfigurement.

The incubation period is at least a week and can be up to many months.

Cutaneous leishmaniasis is generally divided into Old World and New World forms that are determined by the location and causative species. In the Old World the disease is distributed around the Mediterranean, in north, east and Sub-Saharan Africa, the Middle East, Central Asia and parts of India. In the New World it is seen in south Texas, Central America and in all countries in South America except Chile and Uruguay (see Figure 3).

The skin lesions have local common names such as oriental sore, Baghdad boil, pian bois, chiclero's ulcer, uta and espundia (mucocutaneous leishmaniasis). A summary is given in Table 2.

**Diagnosis**

Leishmania are easy to detect by light microscopy but the correct specimen is required. For visceral leishmaniasis the most sensitive test is microscopy of splenic biopsy, but bone marrow or lymph node can also be used. The organism can also be cultured *in vitro* or in hamsters. There are several serological tests available, perhaps the most innovative is an immuno-chromatographic dipstick test for a specific antigen of *L. infantum* used in India. There is a test for cell-mediated immunity, the Leishmanin test, which involves injection of antigen intradermally and is analogous to the Mantoux test for tuberculosis. It has no role in the diagnosis but can be used at the population level to map disease patterns and outbreaks.
Table 2. Species, distribution, vector, reservoir and clinical picture for leishmania species causing cutaneous disease. (from Bell^5^)

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Vector</th>
<th>Mammalian reservoir</th>
<th>Clinical picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. tropica</td>
<td>India, Pakistan, Afghanistan, Mediterranean</td>
<td>Phlebotomus</td>
<td>Humans (Anthropodonic cutaneous leishmaniasis)</td>
<td>Oriental sore, Urban CL, Recidivans CL</td>
</tr>
<tr>
<td>L. major</td>
<td>North Africa, Middle East, Central Asia</td>
<td>Phlebotomus</td>
<td>Various desert rodents such as gerbils and rats</td>
<td>Oriental sore, Rural CL</td>
</tr>
<tr>
<td>L. aethiopica</td>
<td>Ethiopia, Kenya</td>
<td>Phlebotomus</td>
<td>Hyraxes (small rodents)</td>
<td>Oriental sore, Disseminated CL</td>
</tr>
<tr>
<td>L. braziliensis</td>
<td>Central and South America</td>
<td>Lutzomyia</td>
<td>Forest rodents, Sloths</td>
<td>Mucocutaneous CL, Pan Bois, South American CL</td>
</tr>
<tr>
<td>complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mexicana</td>
<td>Central America, Lutzomyia Brazil, Venezuela</td>
<td>Forest rodents</td>
<td>Chiclero's ulcer, South American CL</td>
<td></td>
</tr>
<tr>
<td>complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. peruviana</td>
<td>Peruvian Andes, Lutzomyia Argentina</td>
<td>?dog</td>
<td></td>
<td>Uta</td>
</tr>
</tbody>
</table>

Note: This table is an approximate guide only.

Traditionally, diagnosis of cutaneous leishmaniasis is done through either a “touch smear” (from a biopsy) or a slit-skin smear as in the diagnosis of leprosy. This is best taken from the edge of the lesion. Culture can also be used but serology is generally not reliable. None of these tests have a high sensitivity so a combination is often recommended in areas of high prevalence.\(^\text{10}\) There is some promising progress made with diagnosis by polymerase chain reaction (PCR) technology.\(^\text{11}\)

Amastigotes are usually seen within tissue macrophages (2-3μm across) and are also known as Leishman-Donovan bodies. They have characteristic large nuclei with an extra dark kinetoplast in the cytoplasm.

**Epidemiology**

It is estimated that globally there are about half a million new cases of visceral leishmaniasis and 1.5 million new cases of cutaneous leishmaniasis each year.\(^\text{10,12}\) It affects all ages. The most eastern regions in distribution maps for leishmaniasis arc; Bangladesh, eastern India and northern China for the visceral form and the central Asian republics for the cutaneous form. However, there were 46 cases reported in East Timor following the conflict there in 1999.\(^\text{13}\) There have been no other cases reported in the literature in South East Asia or the Pacific, although potential vectors are present in some countries in these regions.\(^\text{4}\) There have been reports of travellers returning to Australia with leishmaniasis, the most recent report being from Darwin in 1998.\(^\text{14}\)

Leishmaniasis is an opportunistic infection acquired by people living with AIDS. The AIDS pandemic in regions of high incidence of leishmaniasis together with increased migration to the rural-urban fringe has led to an increase in many countries. Co-infection with HIV and Leishmania is emerging as an important new disease complex with clinical, epidemiological and economic implications.\(^\text{15}\) The ongoing conflict in Southern Sudan, in combination with the increasing HIV prevalence in that region has led to an epidemic of visceral leishmaniasis in that country in recent years.\(^\text{16}\) In Darwin, we have had an increase in the number of refugees arriving from Sudan and kala-azar should be considered in the differential diagnosis of unexplained fever with hepato-splenomegaly.

**Treatment**

The mainstay of treatment for both visceral and cutaneous leishmaniasis has been the pentavalent antimonial drugs such as sodium antimony gluconate and meglumine antimonate. These
need to be given parentally and are associated with numerous and serious side-effects and with the development of resistance. Newer drugs such as liposomal amphotericin B and oral miltefosine (a cytotoxic agent) have been used in visceral leishmaniasis with excellent results. Many cutaneous leishmaniasis lesions heal without treatment, however size, position or cosmesis may necessitate intervention. Physical removal such as curettage or cryotherapy has been used in the past together with the antimonials. Miltefosine has been shown to be effective and for some species the antifungal azoles (in particular fluconazole) have shown promising results. Local treatments such as intra-lesional antimonials, paromomycin ointment and Imiquimod (a wart treatment) have also been shown to be effective.

Control

The essential step in the control of leishmaniasis is to separate humans from sandflies or the animal reservoir. In the past, malaria control programs have had a large impact on the transmission of Leishmania due to the impact on sandfly populations. Successful sandfly control has been achieved through household spraying (with pyrethroid sprays) and with the use of bed nets either treated with pyrethroids or untreated. Control of the mammalian reservoir by environmental change, or culling or treating local dogs has been successful in some settings.

Leishmaniasis has always been considered a good candidate for vaccine development. Milestones for progress here have been the recognition of the role of CD8 cells in the immune response, the Leishmania genome project, the funding boost from the Bill and Melinda Gates foundation, and WHO support for a program to resurrect “leishmanisation”, whereby infants are inoculated with L. major to develop a sore and thereby prevent cutaneous disease later in life.

Risks of transmission to humans in the NT

In the Northern Territory, the risk of spread from kangaroos to the human population is indeed very small. The known sandfly vectors for transmission to humans do not exist in Australia and there are no phlebotomine species that are pests of humans in Australia. Phlebotomine attack on humans in the Australasian region is very rare overall and transmission of diseases of humans or domesticated animals in the region has been regarded as most unlikely. However there could be a local, but previously undetected, Leishmania in Australia being transmitted to local hosts that do not exhibit symptoms of disease. There are a number of phlebotomine sandflies species in Australia that could be local vectors. This includes the genera Australophlebotomus, Sergentomyia and Idiophlebotomus. This last genus is known from a single species which bites bats and has been found in caves.

There are 8 species of Australophlebotomus, of which one, Australophlebotomus brevifiloides has been recorded once as biting a human. The 2 most abundant species dominate in the south of Australia and favour moderate or lower rainfall environments. Australophlebotomus brevifiloides normally bites small animals, birds and reptiles. It has been recorded in the NT, mostly from the Barkly and south of (and including) Katherine.

Sergentomyia has the most species (24), with S. queenslandi and S. englihsae the 2 most abundant and widespread species. S. queenslandi is the most frequently collected phlebotomine species from Australia, with the biggest densities found in the northern half of Australia from low rainfall areas, often associated with rocky outcrops. This species has been found in the Darwin region including Beatrice Hill, Howard Springs and Mudginberri. Adults have been collected from burrows of small reptiles and it is reported to feed readily on lizards. However human attack by S. queenslandi is unknown in Australia.

It is interesting to postulate why this disease has appeared only in the Red Kangaroo and how it was transmitted to these animals, at least one of which was geographically isolated from the others. The Red Kangaroo is an exotic species in the Top-End and, given that it probably exists under some ecological stress, may lack immunity to local pathogens, leaving it susceptible to disease from a previously undetected local Leishmania. If this is the case, and presuming that the local parasite has been around since at least European settlement of the
NT, it appears not to cause illness in its natural local host, nor indeed spread to humans or domestic animals.

Studies are being planned to ascertain what arthropods are biting the kangaroos and whether there are local animal reservoirs of Leishmania. The evidence that reptiles are preferred hosts for some of the Australian phlebotomines and the collection of adult sandflies from reptile burrows indicates that reptiles, and particularly goannas and their burrows, are good candidates for the investigation of a local Leishmania in the Top End.

Summary

Leishmaniasis is an exotic protozoal disease which is transmitted between humans or between animals and humans by phlebotomine sandflies. The recent discovery of a possible Leishmania species in captive kangaroos in the NT has raised the possibility of an emerging pathogen which could impact on human health. Even though a theoretical risk exists, the lack of human biting phlebotomine sandflies in Australia, and the lack of any evidence of spread between other marsupials or mammals, suggests that the risk to human health in the NT is minimal. Nevertheless, clinicians should keep the theoretical risk in mind when assessing non-healing ulcers. It is also worthwhile being aware of the possibility in the context of refugee health, given that both internecine conflict and the HIV pandemic have led to an increase in leishmaniasis in other parts of the world.

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References