The leishmaniases - survival and expansion in a changing world. A mini-review

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The number of cases of visceral and cutaneous leishmaniasis is increasing globally at an alarming rate irrespective of the region and the leishmaniases are amongst the top emergent diseases in spite of control measures. In the present review attention is drawn to some of the reasons for this. The leishmaniases have expanded beyond their natural ecotopes due to the ecological chaos caused by man and this in turn affects the levels of his exposure to the vectors. Examples of how different phenomana (such as war, civilian migration, immuno-suppression caused by medication and viral infections, globalization of work and leisure and transmission outside endemic areas) contribute to the spread and increase of the disease are discussed.

The leishmanias have evolved over many millions of years under selective pressures that depended on natural ecological changes. These may have been rapid such as the havoc caused by storms, flooding, hurricanes, and volcanic eruptions that disrupted host and vector relationships. Or they could have been slower due to global climatic changes, such as the ice ages, in which vast amounts of water were locked away leading to drastic reductions in rainfall and the consequential extension of arid regions. Or even slower due to the movement of tectonic plates resulting in the separation of enormous land masses or the formation of gigantic mountain barriers. However, none of these situations approaches the speed and extent of the ecological damage wrought by man during the past 2000 years culminating in the devastation of enormous areas of natural habitats in periods as short as 50 years, global warming and the rapid movements between different ecotopes, at lightening speeds due to the transportation revolution. In the present paper I plan to use different examples to illustrate how the epidemiology of leishmaniasis has expanded into territories that are no longer related to natural phenomena but to man's own activities.

If we take a look at the recent history of the *Leishmania* there is are points in time at which man inadvertently domesticated some species, as he did other animals during the course of his social evolution. There seems to be no doubt that all *Leishmania* species have a zoonotic origin. Thus the first changes associated with man are the adaptation of *L.* (*Leishmania*) *donovani* and *L.* (*L.*) *tropica* to anthroponotic cycles, which in terms of the evolution of the genus must have been an extremely recent event. Some epidemiological observations indicate that in some regions *L.* (*L.*) *tropica* is still a zoonosis and experimental evidence (Kamhawi et al. 1995, Svobodova et al. 2003) suggests that rodents could be reservoirs.

During the last 20 years or so we have seen an increase in the number of cases of all forms of leishmaniasis throughout the world and this has led to it being considered by many experts as an emergent disease in some areas and in others as a re-emergent one (Ashford 2000). Attention has been drawn to many factors that contribute to the expansion of the leishmaniases and Dujardin (2006) considered that environmental changes, immune status, and treatment failure constituted the three major risk factors responsible for the emergence, remergence, and spread of the disease. There is no single reason for this and it varies from region to region. In some cases it is the stress and upheaval caused by civil war, such as in the Sudan (Ashford et al. 1992, Moszynski 2002) and in others it is uncontrolled migration to urban areas due to economic pressures (Dye & Williams 1993) or again war (Hewitt et al. 1998, Rowland et al. 1999). The number of cases in professionals has also been increasing in recent years, especially in members of the armed forces who are sent to endemic areas (Hepburn et al. 1993, Hyams et al. 1995). Air travel is now much cheaper and many thousands of holiday makers in searching for the sun or exciting places end up in endemic areas and contract leishmaniasis (Manfredi et al. 2001, Scope et al. 2003). There are also indications that global warming is changing the geographical distribution of vectors which could lead to transmission in hitherto non endemic regions (Kuhn 1999, Peterson & Shaw 2003).

A POSSIBLE COMMON FACTOR INFLUENCING OVERALL INCREASES IN CASES OF LEISHMANIASIS

Is there a common group of factors in all the above events? In my opinion there is and it is an infinite variety of different reservoir/man vector contacts which modulate the level of immunity, determining whether or not there will be clinical disease. This latter concept is extremely important because there is a growing amount of

evidence indicating that parasites persist for life (le Fichoux et al. 1999, Riera et al. 2004) and that due to some breakdown in the immune system disease may result at a later date (Walton et al. 1973). Sand fly biting rates may vary from extremely sporadic to almost constant depending on the region and the habits of the individual can also be important risk factors (Caldas et al. 2002). It is has been shown experimentally in mice that the bites of uninfected sand flies can infer protection (Kamhawi et al. 2000) against subsequent infection. This suggests that high levels of sand fly bites in endemic areas could be protective and result in asymptomatic infections in man. Thus given the globalization of everything from commerce to war it is quite conceivable that people from all walks of life can find themselves in situations where their first experience to sand fly bites could include infected ones. Examples of some such situations are given below.

SOLDIERS IN TRAINING AND WAR

Members of the armed forces are perfect targets for the hungry infected sand fly when they are sent to tropical regions. Most are residents of countries or regions where leishmaniasis is not endemic. Brazilian soldiers are sent on the jungle war training course near Manaus, Amazonas and over the years many have become infected (de Oliveira Guerra et al. 2003). There are, however, other records of similar types of infections in such regions such as Belém, Pará (Silveira et al. 2002) and Pernambuco (Brandão-Filho et al. 1998). The American Army's jungle training centre in Panama (Takafuji et al. 1980, Sanchez et al. 1992) has similarly resulted in cases of cutaneous leishmaniasis amongst troops for many years.

However, soldiers are now becoming infected in war zones in different regions of the world. There was an outbreak of zoonotic cutaneous leishmaniasis in 36/80 Jordanian soldiers involved in a mine sweeping operation after the Arab-Israel conflict (Jumaian et al. 1998). In the Desert Storm war American personnel were infected with a parasite identified as *L. (L.) tropica* that produced visceral symptoms (Magill et al. 1994). Because of this all soldiers involved in the campaign were banned as blood donors for one year. This raises important questions related to blood donors and the risk of transmission via blood transfusions. There is evidence that patients cure and that their blood can become parasite positive again after 1 to 30 years (Guevara et al. 1993, Delgado et al. 1996) as well as the reactivation by immunosuppressive drugs (Scatena et al. 2003). Similarly the fact that there is strong evidence that transmission of visceral leishmaniasis (VL) amongst HIV positive drug users in Europe (Desjeux & Alvar 2003) is by contaminated syringes again indicates the danger of contaminated blood. These examples emphasize that there must be asymptomatic cases of leishmaniasis in non-endemic areas and at present blood banks do not screen for leishmanial infections.

CIVILIANS IN WAR AND SOCIAL UNREST

There are many examples of how war and social pressures can results in outbreaks that reach epidemic levels. One of the more recent ones of almost unimagin-

able proportions was the epidemic of VL in the Southern Sudan, known as the Western Upper Nile (WUN) region (Seaman et al. 1996). People found themselves in the middle of a civil war which impeded diagnosis and treatment. In trying to escape they moved to new areas where there was no food and this led to high levels of malnutrition. It has been estimated that from 30 to 40 thousand people died in this epidemic (Davidson & Croft 1992) and that it is still rampant (Moszynski 2002). One of the alarming consequences of this besides the high death rate is that it has been estimated that 50% of the treated cases develop post dermal kala-azar (PKDL) which normally cures spontaneously (Zijlstra et al. 2003). However, such cases could serve as reservoirs of infection.

Less spectacular, but equally disturbing are the outbreaks in the poorer slum regions of some Brazilian towns such as Santarém, Pará and Terisinha, Piauí. Although the disease is endemic in the rural areas of these regions it was not considered to be an urban disease. There are, however, now records of VL in other cities such as Belo Horizonte, Minas Gerais (Silva et al. 2001) and Araçatuba , São Paulo (Camargo-Neves et al. 2001) The threat of the urbanization of VL in many other regions of Brazil is real. The reasons are unknown but it is most likely associated with an adaptation of the principal vector, *Lutzomyia longipalpis*, to the urban environment.

During war civilians become fugitives in their own country or end up as refugees in neighbouring countries where they live precariously. The war in Afghanistan was no exception to this phenomena and the result were epidemics of *L. (L.) tropica* in Kabul (Ashford et al. 1992) and in refugee camps in Pakistan (Rowland et al. 1999).

IMMUNODEFICIENT INDIVIDUALS

Another scenario that we are presently witnessing is infections in immuno-suppressed individuals. This suppression may be due to viral infections, such as HIV, which have led to an increase in the number of recorded cases of VL in countries where the disease was rare such as France, Italy, Spain, and Portugal (Desjeux & Alvar 2003). Epidemiological evidence indicates that within this group transmission is highest amongst drug addicts due to contaminated syringes. There are ever increasing numbers of organ transplants throughout the world whose recipients are obliged to take immunosuppressive drugs for the rest of their lives. The risks to such individuals are multiple. Clinical leishmaniasis may result from the activation of occult infections in patients undergoing organ transplants (Berenguer et al. 1998, Morales et al. 2003), contaminated organs or higher levels of susceptibility to natural transmissions.

Besides exacerbating occult infections immunosuppression can alter the clinical symptoms more commonly associated with a particular species of *Leishmania*. For instance in HIV patients *L. (Viannia) braziliensis* can cause visceral infections (Silva et al. 2002) or disseminated skin lesions (Coura et al. 1987). Transmission levels may also increase when the vector feeds on immuno-depressed individuals with high parasitaemias (Molina et al. 2003).

GLOBILIZATION AND BLOOD TRANSFUSION

The globalization of world commerce has led to an unprecedented movement of goods and individuals between countries and continents. Inevitably this is associated with members of professional groups ranging from company employees, who may or may not be accompanied by their families, to servicemen and sometimes their families spending varying periods of time in endemic leishmaniasis areas. Besides this there is also an intense exchange of tourists in search of exciting holidays in tropical countries. A literature search reveals many cases of leishmaniasis associated with such events and it is not my intention to review them in detail. However, I will use one or two examples to emphasize how globalization is changing the distribution of the disease and the dangers associated with this.

One of the gravest clinical problems is the occurrence of leishmaniasis, especially the visceral form, in regions where health workers do not expect it to occur such as say Scotland. Many years ago there was a case of a Scottish mother who after having a caesarean operation began to loose weight accompanied by periods of fever. Miraculously she was diagnosed as having VL but how could a women who had never left Scotland have contracted the disease? A clinical inquiry revealed that she had had a blood transfusion during surgery and they traced the donor as being an Asian seaman who was in transit. Of 506 healthy French blood donors 76 had a positive leishmanial serology in a specific Western blot test and parasites were isolated from two (le Fichoux et al. 1999).

The above examples point to a serious problem – transmission during blood transfusion. There are only a few cases registered in the literature but the danger is real and increasing. No blood bank screens for leishmanial antibodies and even if it did the level of efficiency of detecting healthy carriers with any of the existing tests is to say the least dubious. In the absence of a suitable test one measure is to eliminate any individual who has visited an endemic area even during their holidays but what about those who live in endemic areas? In Brazil there was a chance in the past that at least some leishmaniasis carriers would be detected by the obligatory *Trypanasoma cruzi* screening test. However, the specificity of the latter test has been improved to eliminate cross reactions with such diseases as leishmaniasis! The question is how does one deal with this problem which effects highly populated regions of South America and the Mediterranean region? Treatment of the blood with a preservative is a possibility that may have to be considered.

Of 1194 volunteer blood donors in Natal, Rio Grande de Norte, Brazil, 9% were serologically positive for VL, increasing to 25% in a periurban kala-azar focus (Luz et al. 1997). However, 37% of the multiply transfused hemodialysis patients for the same city were also positive whereas in Rio the VL antibody prevalence was 7% in the same patient group. This strongly suggests that transmission is occurring via blood transfusions.

One can only conclude that many people are probably becoming infected via blood transfusions. In countries, such as Brazil, where leishmaniasis is endemic these cases would probably go unnoticed especially in endemic areas.

DOGS AND TRAVEL

Another factor associated with travelling families is that when ever possible they take their dogs with them and long periods of quarantine between trips are no longer necessary with the advent of more efficient vaccines for canine diseases and animal health passports. The incubation period of canine visceral leishmaniasis (CVL) is variable and clinical symptoms can appear after leaving the endemic zone. This means that infectious dogs may go unnoticed for long periods and could serve as sources of infection for sand flies and blood based products or mechanical transmission.

In the US there are both imported and autochthonous cases of CVL. Cases that are considered to be autochthonous have been recorded in Oklahoma in 1980; Kansas in 1982; Ohio in 1988; Michigan in 1989; and Texas and Alabama in 1991. In a survey of 11,010 dogs from Hunt Club Kennels performed between 200 and 2001 by CDC a total of 71 animals from 21 states had positive IFAT antibody titres. In this survey parasites were isolated from dogs from the states of New York, Michigan, Virginia, Kentucky, Alabama and Georgia and the Canadian State of Ontario (Enserink 2000). So far there is no evidence of sand fly transmission but they are present in Texas and some eastern states. It was thought that sand flies did not occur in Upper New York state where the most recent US outbreak of CVL occured. However, *Lu. vexator* was found (Ostfeld et al. 2004) to be abundant in the neighbourhood of the Millbrook Kennel and must be considered as a potential vector. Ever since the first recorded outbreak in 1980 in Oklahoma the methods of transmission have been surrounded by mystery. Clearly vector transmission must be considered especially as sand flies are known to occur in the eastern states were many cases have been recorded. Besides this though it is possible that different methods of mechanical transmission may occur. Amongst those that must be considered are biting, tattooing, contaminated needles and blood transfusion. Owens et al. (2001) noted that 3/7 foxhounds who had received packed red blood cells from seropositive dogs became infected. They concluded that foxhounds should not be used as blood donors.

In the recent out break in upper New York state infections were only found in foxhounds and not in Beagles or Basset hounds from the same kennel. The association of VL with foxhounds raises a number of points. Firstly these dogs travel and could be exposed to sand fly bites on such occasions. Besides this though they are gregarious and often kennelled in large numbers that result in fighting. It could well be that all these factors, as well as the stress of hunting, contribute to them being the race in which most cases of VL have been recorded. The problem is not limited to the US. A study in Holland (Slappendel 1988) showed that 92 dogs imported from the Mediterranean area were infected but there were also two autochthonous cases. One of the latter cases was

the puppy of a bitch who had become infected in Spain (Diaz-Espineira & Slappendel 1997). The review by (Slappendel & Teske 1999) should be consulted for a fuller review of the CVL records outside the endemic areas. All these observations raise the question as to whether or not such transmission is occurring in endemic areas? For instance many of the social characteristics of foxhounds are similar to those of stray dog packs that are common in endemic areas of Latin America.

Perhaps no other country in the world has invested more money in attempting to control CVL than Brazil. However, all their efforts have failed for many different reasons but one of the most important lessons that can be learnt from these failures is that the elimination of infected dogs is neither effective nor socially acceptable. Of the presently available methods those that kill sand flies that feed on infected dogs, such as insecticide impregnated collars (Killick-Kendrick et al. 1997), are socially the most acceptable and economically feasible. Field trials in Iran (Gavgani et al. 2002) showed that seroconversion rates for visceral leishmaniasis antibody was lower in the inhabitants and dogs of the villages were collars had been used. Observations are still required to define how long collars must be used to eliminate transmission in an endemic area, the cost and the effect of wild dogs. It must be remembered that there is no point in beginning any program unless it is financially sustainable and that a vaccine for CVL would almost certainly be more cost effective.

TRANSMISSION TO MAN OUTSIDE ENDEMIC REGIONS

On diagnosing an autochthonous case near Aachen, Germany, Bogdan et al. (2001) reviewed the literature and noted that there were sporadic cases in Central and Northern France (Guilhon 1965) as well as in Switzerland (Mazzi 1976) and Austria. The interesting point raised by these cases is that it was impossible to determine how these individuals had become infected. The occurrence of infected dogs is not uncommon in these areas (see section on *Dogs and Travel*) and some mechanical method, such as contamination from infected dog skin or urine, cannot be ruled out. Amastigotes have been found (Riera & Valladares 1996) in the urine and semen of experimentally infected dogs and it is conceivable that both could be sources of contamination for other animals including man himself. There is an embarrassing record of horizontal transmission between a married couple (Symmers 1960). Initially syphilis was suspected but the husband said this was impossible since he had had no sexual relationships outside his marriage. Unfortunately his wife did not believe him and their marriage ended in divorce in spite of the final diagnosis of cutaneous leishmaniasis. In the case of VL it is possible that transmission could occur during intercourse from infected semen (Riera & Valladares 1996).

Vertical transmission in man appears to be rare. An interesting case was recorded (Meinecke et al. 1999) in a 15 month old boy whose mother, during her holidays, had visited endemic visceral leishmaniasis areas of Europe several times before her pregnancy. She had a posi-tive skin test and specific antibodies were demonstrated in a Western blot reaction. She had never had any clinical symptoms and the authors concluded that most probably she had a sub-clinical infection that was reactivated during her pregnancy and it was this that resulted in the congenital transmission. The authors considered that this method of transmission is rare and could only find eight other published cases. However, these occurred in endemic regions but in areas that were considered as unsuitable for the vector.

However, sand fly transmission must always be considered, even in non-endemic regions. In Switzerland *Phlebotomus perniciosus*, a known VL vector, is common. It also occurs in France as far north as the regions of the Pays-de-Loire and the Ile-de-France, in which Paris is situated, and *P. mascittii* has been found in the department of Calvadas that borders the English Channel (Rioux & Golvan 1969). More recently *P. mascittii* was found in the region Aachen region, Germany (Naucke & Pesson 2000). Although this species has never been implicated as a vector it must be considered as a potential one. It is quite possible that other sand flies, besides the classical ones, could be vectors in certain situations. Thus in Colombia it has been shown (Travi et al. 1990) that *Lu. evansi* is an important VL vector in the absence of *Lu. longipalpis*.

CHANGES IN THE BEHAVIOR OF THE SAND FLY VECTORS

Transmission of *Leishmania* by sand fly species other than those that are associated with the feral enzootic cycle may occur. For instance if an infected mammal enters an area where there is a susceptible sand fly the latter will take over the function of the classical vector. Volp nad Myskova (2007) reviewed this phenomenon and called such sand flies permissive vectors. Such a sand fly in Brazil is *Lu. longipalpis* which is a potential vector of *Leishmania* such as *L.(L.) amanzonensis* and *L.(V.) braziliensis*. *Lu. flaviscutellata* has recently been found infected (Fouque et al. 2007) with *L.(V.) guyanensis* and infections of *L.(L.) amanzonensis* have been found (Tolezano et al. 2007) in dogs from a CVL region where the principal sand fly was *Lu. longipalpis*. The latter suggests that *Lu. longipalpis* may be transmitting two *Leishmania* or that *Lu. flaviscutellata* is changing is habits. What is emerging are infections in mammals and sand flies that do not follow the well studied sylvatic enzootic cycles. In the past such events happened and it is ironical that the sand flies that became domesticated also became vectors of both visceral and cutaneous parasites.

In Brazil there are records of human cases of VL in areas considered to be non-endemic that have baffled the public health authorities. Of these two from Greater São Paulo (Iversson et al. 1979, 1982) are particularly important because there was evidence of sub-clinical infections in children from the same neighbourhood as one of the sick children. Neither *Lu. longipalpis* nor infected dogs were found but the authors considered that the epidemiological data suggested some form of natural transmission. Very recently a naturally infected cat was found (Savani et al. 2004) in Cotia which is close to

São Paulo city and so far *Lu. longipalpis* has not been found. The absence of *Lu. longipalpis* suggests that transmission may involve other sand fly species.

A general picture is gradually emerging that suggests that transmission may not necessarily be associated with the accepted vector species. Further epidemiological and experimental data is needed to confirm this in the areas where accepted vectors are absent but clinical cases occur.

THE FUTURE

It is difficult to predict what will happen but the events reviewed in this paper indicate that leishmaniasis is becoming a global disease. There is now evidence that both vertical and horizontal transmission by mechanical means are occurring for VL. Because of this the risk of infection outside the endemic areas is increasing. However, the possibility of sand fly transmission must always be considered. The challenge is how to control so many different *Leishmania* that have enzootic cycles? The recent finding (Karunaweera et al. 2003) that the aetiological agent of cutaneous leishmaniasis in Ceylon is *L. (L.) donovani* in the absence of the visceral disease raises, yet again, the question of whether or not there are animal reservoirs of those *Leishmania* that are classically considered to have none.

Only when we have a better understanding of the genetic diversity of the *Leishmania* and the reservoirs involved in each species' enzootic cycle will it be possible to evaluate which control method or methods are most likely to succeed.

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