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# A protozoologist in the Amazon – conversation between Ralph Lainson (RL) and Manoel Soares (MS)*

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Un protozoologista en la Amazonía – una charla entre Ralph Lainson (RL) y Manoel Soares (MS)

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**MS** - Dr. Lainson, how did you come to establish a research project on leishmaniasis in the Amazon Region of Brazil?

**RL** - This is a long story Manoel, commencing in 1959 when I was working in the Department of Medical Protozoology of the London School of Hygiene and Tropical Medicine (LSHTM), which at that time was under the direction of the renowned protozoologist and malaria specialist, Prof. P. C. C. Garnham.

I was perfectly content with my work there, and particularly with life in London, but one day Garnham called me to his laboratory. "Lainson," he said, "you are not going to learn much about tropical diseases here in London, so I propose sending you and an entomologist to British Honduras in Central America. You will have three years to discover the reservoir hosts and the vector of *Leishmania mexicana*. This parasite is responsible for the cutaneous leishmaniasis of people working in the forest, so this is where you will be working most of the time." A few weeks later, entomologist Strangways-Dixon and I were organizing a modest little laboratory in the Department of Agriculture in a remote area of British Honduras (now known as Belize).

During our first year, we trapped a number of small forest mammals, and a few larger ones were purchased from local hunters. All the animals were sacrificed. Blood and the triturates of liver and spleen were cultured in blood – agar culture medium (NNN) and inoculated intradermally into hamsters and laboratory mice, but to our great disappointment, *Leishmania* was not isolated. As we later discovered, this result was not surprising because *L. mexicana* proved to be essentially dermatropic in the wild animal reservoir hosts, and it very rarely invades the blood or the viscera. On the positive side, however, the vast number of sand flies captured from human bait provided us with a valuable list of highly anthropophilic species.

Source: Instituto Evandro Chagas, [between 1964-1969]

**Figure 1** — Ralph Lainson and Jeffrey Shaw during preparation of sheets for leishmaniasis study in the 1960s

During our second year, hurricane "Hattie" struck British Honduras, destroying much of the capital Belize and resulting in the loss of over 400 lives. It also toppled much of the forest in our study area and left us wondering if this meant the end of our project. Eventually, however, we managed to retrieve a fair number of our precious traps from the mass of fallen trees and recommenced trapping. To our great surprise, the number of small rodents captured increased considerably, probably due to a shortage of food in the damaged environment that resulted in their increased interest in the bait of our traps.

One day whilst examining some of these rodents, I noted a small nodule on the base of the tail of one animal, and I cleaned the area, incised it and prepared a Giemsa-stained slide of the exudate. Expecting to see nothing more than bacteria or fungal spores in the smear, I saw instead a large number of beautiful little amastigotes of *Leishmania*; this moment in my life I shall never forget! The parasite was isolated both in culture and in the skin of hamsters inoculated intradermally with the triturated material from

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the lesion; it was identified as *L. mexicana*. Subsequently we were able to record similar infections in four different species of small forest rodents.

On our return to the laboratory one night, after capturing sand flies with human bait, we fed a number of the flies on the skin lesions of hamsters infected with a strain of *L. mexicana* isolated from one of the wild rodents. After feeding, each fly was housed separately in a small glass tube for one week, after which all were again fed, individually, on a volunteer. The bite of one of these sand flies, identified as *Lutzomyia* (*Psychodopygus*) *pessoana*, transmitted the parasite to the volunteer.

On my return to London, I could think of little else other than the possibility of carrying out a similar investigation on *Leishmania braziliensis*, the major causal agent of cutaneous and mucocutaneous leishmaniasis in Brazil and neighbouring countries. Thus, in 1962, I visited the Instituto Evandro Chagas (IEC) in Belém (Pará State, northern Brazil) to discuss, with the Director, the late Orlando Costa, the possibility of a collaborative programme on leishmaniasis in the Amazon Region.

Present at our discussion was the Institute's virologist Dr. Otis Causey who, I was delighted to hear, was trapping forest mammals for the detection of arboviruses. I showed him some photographs of lesions caused by *L. mexicana* on the tails of rodents, and he was impressed by the similarity that they had with lesions he had seen on the tails of a number of the rodents he had captured. He had thought, however, that they were probably due to bacterial or fungal infections of damaged tails. I suggested that he should make Giemsa-stained smears from the next lesions he saw to determine if they contained *Leishmania* amastigotes. Just a few days later, he showed me the stained smear of a tail lesion of a rodent (*Oryzomys capito*) that had been trapped in the nearby Utinga forest, and it was rich in amastigotes!

This discovery prompted the Wellcome Trust in London to immediately offer me a grant to study leishmaniasis in the Department of Parasitology at the IEC. Provisionally, the grant was for three years with the promise of extension should the results of our research warrant it; it so transpired that the Wellcome Parasitology Unit (WPU) continued its studies until my retirement in 1992!

Shortly before my return to Belém to organize the WPU's laboratory and my own accommodation, I met Dr. Jeffrey Shaw, who had just returned to the London School of Hygiene & Tropical Medicine from Panama, where he had been working on the life cycle of *Endotrypanum schaudinni*, a peculiar intraerythrocytic flagellate of sloths. I noted a lesion on his neck that had all the aspects of cutaneous leishmaniasis. He had received no diagnosis after consulting several doctors, so I suggested that we should prepare a stained smear. My suspicion was confirmed when we detected amastigotes of a *Leishmania* species, which we later named *Leishmania panamensis*, in the smear. This result led to a conversation on neotropical cutaneous leishmaniasis, and I asked Jeffrey if he would like to join me in Belém. He liked the idea, and, in fact, he worked with me until the WPU's project ended in 1992!

**MS** - What was our knowledge of *Leishmania* and cutaneous leishmaniasis in Brazil when you both arrived to work in the IEC?

**RL** - The general opinion was that *L. braziliensis* was the cause of all forms of cutaneous leishmaniasis in this country, and as a result, some workers in the Institute (Guimarães & Costa, 1964) actually referred to the parasite of *O. capito* by this name. Following its isolation from a variety of rodents and some marsupials, this parasite was shown to be very different from *L. braziliensis* we obtained from local cases of cutaneous and mucocutaneous leishmaniasis.

Its amastigotes were much larger, and inoculation of the parasite into the skin of hamsters and laboratory mice rapidly produced huge tumours containing an enormous number of these large amastigotes. The parasite was easily cultured in the simplest of blood-agar culture media (NNN) but, in contrast, *L. braziliensis* multiplied very slowly in the skin of hamsters and mice, needing several months before producing a small lesion containing scanty, small amastigotes. Its growth in NNN culture medium was also slow, and attempts to maintain the parasite in vitro frequently failed.

In view of these striking differences and its behaviour, similar to that of *L. mexicana* of Central America, the *Oryzomys* parasite was named *L. mexicana amazonensis*, and later this was amended to *L. amazonensis*. Finally, biochemical, serological and molecular techniques clearly indicated the necessity of a new specific name.

Slowly, we recorded cases of human infection with *L. amazonensis*, usually in the form of single cutaneous lesions that responded well to antimonial treatment, and we also showed that this parasite was responsible for the rarer "anergic, diffuse, cutaneous leishmaniasis" (ADCL) in individuals with defective immune systems. This condition is extremely disfiguring and extremely resistant to the usual treatments.

Isolates of leishmanial parasites from a large number of patients and a wide variety of wild mammalian reservoir hosts gradually revealed the presence of other species of *Leishmania*. Frequently, the parasites were initially discovered in the wild animal reservoir host, and only much later were they found in humans. Based on the development of these parasites in their phlebotomine sand fly hosts, the genus *Leishmania* was divided into two subgenera: *Viannia*, developing in the hindgut, midgut and foregut of the insect, and *Leishmania* restricted to the midgut and foregut. These observations clearly dispensed with the idea that all cases of cutaneous leishmaniaisis in Brazil were due to a single parasite, *L. braziliensis* and, to date, research in the IEC has indicated the existence of at least seven different parasites causing this disease in this country, as follows: *Leishmania* (*Viannia*) *braziliensis*, *L.* (*V.*) *guyanensis*, *L. (V.) naiffi*, *L. (V.) shawi*, *L. (V.) lainsoni*, *L. (V.) lindenbergi* and *Leishmania* (*Leishmania*) *amazonensis*. It is quite likely that others will be discovered in the future.

Any research on the ecology and epidemiology of a disease is a long and costly procedure. We are, therefore,

indebted to the Wellcome Trust for their generous support for nearly thirty years and to the IEC for the facilities provided during this long period, without which these results would not have been possible.

**MS** - One interesting question Dr. Lainson. Why did you name your Unit "The Wellcome Parasitology Unit" and not simply "The Wellcome Leishmaniasis Unit"?

**RL** - Two renowned parasitologists greatly influenced my mode of research – Cyril Garnham and Cecil Hoare. These two men had much in common, principally an intense desire to study all kinds of protozoal parasites in all kinds of animals!

As a result, I consider myself a protozoologist, and not simply a "leishmaniologist" and the Unit was therefore named "The Wellcome Parasitology Unit".

It is significant that in 1968 the WPU registered the first cases of Chagas Disease diagnosed in the Amazon Region of Brazil and indicated the importance of oral transmission of *Trypanosoma cruzi* in this region. Working with the WPU, Michael Miles subsequently showed the existence of different strains (genotypes) of *T. cruzi*, which sparked a period of intense study of these organisms in the Amazon Region. Finally, the WPU published reports on nearly 100 new species of parasitic protozoans in the fauna of Amazonian Brazil, including coccidians, trypanosomes and haemosporidians found in fish, lizards, snakes and caimans. With the very efficient help of Sra. Vânia Araújo and her staff at the IEC Library, I am preparing an atlas listing and illustrating such parasites in the hope that this information will encourage and help students to continue this research. Volume I of the Atlas, already in print, dealt with the Haemosporida of reptiles. Volume II will be on the species of *Leishmania* and *Trypanosoma*, while Volume III will deal with the Coccidia and a miscellany of other parasitic protozoans.

Currently, there is a greatly increased interest in the biodiversity of our planet. Most of us think of this concept in terms of the variety of insects, fish, amphibians, reptiles, birds, mammals and plants. If we include the multitude of parasites found in these hosts, however, the degree of biodiversity will be even greater.

**MS** - What advice can you give to youngsters who would like to become parasitologists in Amazonia?

**RL** - A great many philanthropic entities are reluctant to finance research on subjects that have little or no medical importance. An applicant for a grant therefore usually needs to entitle his or her project in a manner to overcome this problem, just as I did with the official title of "Ecology and Epidemiology of Leishmaniasis in the Amazon Region of Brazil". This is the "bread and butter" title, but usually it is possible to find some time to study other parasites that are unknown in humans! When discussing our programme on *Leishmania* and leishmaniasis in Brazil with the Director of the Wellcome Trust during one of my Annual Reports of our progress, he said to me, "Dr. Lainson, I have seen a paper of yours on parasites in the blood of lizards. How is it you have time to work with lizards? Please remember that your grant is for work on leishmaniasis!". "Certainly", I replied, "but this work on lizards was only done on Saturdays and Sundays". It was not completely true, but the subject was never mentioned again!

Finally, during my time at the School of Tropical Medicine & Hygiene in London, I mentioned to Garnham that many medical students asked why they had to attend my lectures on the morphology and life cycles of parasites in birds, lizards and snakes, and I asked him to suggest my best reply. "Very simple, Lainson", he said. "Tell them that the existence of trypanosomes became known after the first description of a trypanosome in a fish. Toxoplasma was discovered in a little rodent, not in man, and after Sir Ronald Ross described a *Plasmodium* species of a little bird developing in mosquitos, this clearly indicated that mosquitos were the vectors of the *Plasmodium* species causing malaria in man!"

The Wellcome Trust grant to the WPU terminated on my retirement in 1992, but the leishmaniasis and Chagas disease research programs have continued unabated. Dr. Fernando Silveira, a former member of the WPU group, has continued with an excellent series of papers on the epidemiology and clinical aspects of visceral leishmaniasis and the discovery of *Leishmania* (*L.*) *lindenbergi*, while Drs. Aldo and Vera Valente have made observations on the importance of *T. cruzi* oral transmission in Amazonia, where Chagas disease is now becoming a serious public health problem. I am sure that the Wellcome Trust will be pleased to know that the programmes the Trust initiated so long ago are still producing valuable results on the two diseases.

In conclusion, we all hope that the highly productive research of the renowned IEC in Parasitology, Virology, Bacteriology and other subjects of Public Health may continue for many years to come!

Thank you Manoel, for all your help in preparing this little version of "**Ralph Lainson, this is your life!**"

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