MEDICINE

Malaria and some new diseases

São Paulo scientists make Rondonia a forward post for research with a high social impact

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PHOTOS EDUARDO CESAR

Research into tropical diseases is experiencing an unique moment in Rondônia. Yes, the old federal territory, strongly colonized by immigrants from the south of the country in the Sixties and Seventies, raised to the status of a state only 21 years ago, has been producing some cutting edge studies, when the subject is malaria, leishmaniasis and other disorders, old or emergent, transmitted to man by a host of insects, mosquitoes and ticks infected by bacteria, viruses and parasites of all sorts. It was, for example, on the banks of the Madeira river, at Portochuelo, a district of Porto Velho, one hour away by boat from the urban area of the capital of Rondônia, that researchers from the Institute of Biomedical Sciences of the University of São Paulo (ICB/USP), which for five years has been maintaining a forward post for studies in the hinterland of the state, and from the Center for Research into Tropical Medicine (Cepem), of Porto Velho, obtained, in 1999, the confirmation of an old suspicion about one of the characteristics of malaria in Brazil that makes it difficult to control this endemic disease.

The scientists showed, unequivocally, that there are in the Amazon basin asymptomatic carriers of *Plasmodium vivax*, a protozoon that causes around 80% of the cases of the disease in Brazil – the other 20% are caused, in their majority, by the *Plasmodium falciparum*, the most aggressive species of the malaria parasite, which kills between 1 and 2 million persons every year, above all children, in Africa, and, in an almost insignificant percentage, by the *Plasmodium malariae*. The definitive confirmation that there have been and there are victims of *P. vivax* without any malaria symptom

Danger at nightfall: children from the Triunfo, district, in Rondônia, bathing at the time of day that the Anopheles mosquito, which transmits malaria, usually attacks

The red cloth in front of the house: an indication that there is a case of malaria suspected: a sign for the motor bike rider from the town hall to stop and collect blood to be examined

was obtained with the use of a more accurate molecular method than the everyday laboratory test used to diagnose the disease: the PCR (polymerase chain reaction) technique, which amplifies the DNA of the parasites and makes it possible to identify the species which the live protozoons in the sick person's blood belong to, even in the smallest of quantities. The work had an international repercussion and yielded an article in the English magazine, *The Lancet*, one of the most famous medical publications in the world.

Locating asymptomatic malaria cases in Rondônia is perhaps the greatest scientific achievement of the two research centers set up in that state, which carry out studies both jointly and separately. But it is not the only one. The Amazonian nucleus of the ICB has found strong evidence of an as yet unknown species of a protozoon of the *Leishmania* genus, which could be a new causal agent of American tegumentary leishmaniasis, (ATL) and infectious disease that every year attacks the mucous tissues of 28,000 Brazilians, from the north to the south of the country. In Monte Negro, a municipality 250 kilometers to the south of Porto Velho where the base of the ICB operates, researchers also found an extremely high level – one of the highest in the world – of a little diagnosed skin disease, chromoblastomicosis. They also identified a new kind of tick of the *Amblyomma* genus, which is found in terrestrial animals, above all the Brazilian tapirs (*Tapir terrestris*), which may transmit some disease to man. "In Rondônia, almost everything is new, and there is much to be researched", says Erney Plessmann de Camargo, aged 66, the former professor of the ICB, recently appointed as a director of the Butantan Institute and the coordinator of a project that has the objective of surveying the fauna of ticks in Rondônia and to confirm the prevalence in these arthropods of three geni of potentially pathogenic bacteria, *Rickettsia, Borrelia and Erlichia.*

Plessmann is the co-author of the article on the discovery of asymptomatic the malaria plasmodium in Rondônia, along with Fabiana Alves (ICB) and Luiz Hildebrando Pereira da Silva, Cepem's scientific director and a world authority on tropical diseases. A former director of two departments of the Pasteur Institute in France, Luiz Hildebrando bears, possibly, the main responsibility for Rondônia having been put onto the map of Brazilian research. "We believe that these symptom-free persons may show immunity to malaria and act as di-

sease hosts", says the veteran parasitologist, who has dedicated over four decades to science. In the view of the ICB and Cepem specialists' views, identifying and treating the asymptomatic carriers of *P. vixax* is just as important for holding back malaria advance as putting into practice the traditional control measures for this endemic disease: combating the transmitting agent, the so-called vector (in Brazil, female *Anopheles darlingi* mosquitoes infected with the plasmodium) and to medicate as quickly as possible the symptomatic cases. Studying the asymptomatic ones is also a way of understanding the mechanisms involved in the apparent natural immunity acquired by these people, which may be useful for the development of a vaccine against malaria from *P. vivax*, a dream that is at least one decade away, in Luiz Hildebrando's opinion. "The majority of research projects for a vaccine against malaria works with cases of infection caused by *P. falciparum*", he says. Incidentally, the genome of the *falciparum* was recently sequenced by an international consortium of laboratories (*see article on page 40*).

In a pilot venture in terms of the Amazon region, researchers from Cepem swill start before the end of this month the identification and treatment of asymptomatic malaria cases in Vila Candelária, a district of Porto Velho, also on the banks of the Madeira river, though much closer to the city than the Portochuelo district. The riverside community of Candelária, where the tracks of the legendary Madeira-Mamoré Railroad pass, lies ten minutes by automobile from the paved part of the capital of Rondonia – a city with 330,000 inhabitants, spread over a flat area of more than 34,000 square kilometers, 20 times larger than the city of São Paulo – and Candelária is visited at weekends by a floating population of holiday makers coming from the more urban part of Porto Velho.

Doctors make the difference - In the village, where 260 fixed inhabitants live, studies by Cepem show that 30% of the residents carry *P. vivax* in their blood, but they do not have the malaria symptoms (a temperature of up to 40° C and continuous sweating , from two to four hours), not to mention that 40% have both things, the plasmodium and the clinical manifestations. "Treating the asymptomatic may change the fate of malaria", says Mauro Shugiro Tada, from São Paulo, and Cepem's head doctor. Tada moved to Rondonia 17 years ago, with the intention of researching tropical diseases in a center, which was the embryo of his current place of work, in Costa Marques,

Human bait: trainees from USP capture mosquitoes (*featured, Anopheles*) with time of day, temperature and humidity monitored

on the frontier with Bolivia, where, he says, "there is malaria even up the papaya trees".

Talking about malaria in Brazil is actually talking about malaria in the Amazon region, a region that concentrates over 99% of the Brazilian cases of the illness. In 2001, according to partial data from the National Health Foundation (Funasa), there were 340,000 cases of malaria in the country and 85 deaths. In Rondônia, the number of cases lay in the region of 50,000. In 2000, the number of patients in the whole country reached 610,000, with 240 deaths. However bad our health system is – and in the Amazon basin, it is even worse than in other parts of the country –, Brazil's reality is still clearly better than Africa's is, and that mitigates the burden of the endemic diseases over here.

"Our severe malaria is different from the African one", says Luiz Hildebrando, who has already caught the disease in Senegal and is a carrier of *Trypanosoma cruzi*, though without suffering from Chagas disease. "The medical service there is much worse, and the favorite victims of *falciparum* are children, and this frequently causes complications in the brain and death. Here, it is the adults who catch malaria, usually farmers or gold-diggers or people who live on river banks, and problems with the brain are rare". There are few doctors in the rural areas of Amazon basin, but by walking a few kilometers it is possible to find some minimally competent medical attention, says the scientist. In Africa, the walk usually leads nowhere.

In spite of his commitments in São Paulo, Erney Plessman goes to the Amazon forest. "In Rondônia, we have the spirit of a sentinel, to monitor old diseases, the emerging ones and those that are re-emerging", he sums up. "You never know when something different may appear, like a case of Ebola". Until now, there are no confirmed reports of victims of this mysterious virus, which causes a hemorrhagic fever capable of killing the patient in days. The researchers from USP and Cepem are not working in Rondônia with the specific intention of looking for cases of Ebola. But as the natural habitat of the virus is the African and Asian rain forests, it makes sense to think that its existence may extend to Brazil and our tropical forests. Like boy scouts, therefore, they always have to be alert.

Exposed to mosquitoes - Even when he cannot travel, Plessmann knows that the tick project is in good hands, in those of his 40 year old son, Luís Marcelo Aranha Camargo, the coordinator of the ICB's Advanced Research Nucleus. With two kinds of malaria in his curriculum – Monte Negro has a high incidence both of malaria and of American tegumentary leishmaniasis –, Camargo stays the greater part of the year in Rondônia. He comes to São Paulo to give lessons and guidance to his students, on average, every 45 days. In the town of 12,000 inhabitants, where electricity cuts and dead

In Monte Negro, On the banks of the Jamari river, collecting goes on: ticks like the probable new species of *Amblyomma* (above) stick to the flannel

telephones are hardly a novelty, one of his tasks is to coordinate a systematic job of capturing the diseases vectors, mosquitoes and ticks. Often, in the search for a mosquito, the so-called human bait is used: a fleshy uncovered leg is offered to the insect by a member of staff or trainee. The method involves the risk of an insect bite, but it works. The ICB has already captured over 80,000 mosquitoes, and, with other techniques, 10,000 ticks.

It was collecting vectors in the forest that Camargo's team caught samples of the candidate to a new species of tick, extremely similar to *Amblyomma incisum*. The ICB researchers are looking for evidence that ticks, besides mosquitoes, are transmitters still little studied of a number of diseases, old or emerging, to animals and to human beings. In many cases, this relationship is still obscure. In others, it is now known. This is the case of spotted fever, caused by the *Rickettsia rickettsii* bacterium, transmitted to man by the star tick (*Amblyomma cajennense*), common in horses and other wild animals. It is a mistake to think that the disease is present only in the Amazon basin. Spotted fever is an endemic disease even in areas of São Paulo, such as the Campinas area, where it has already caused some deaths.

Besides the candidate for a new tick, Camargo's permanent team in Monte Negro and Jeffrey Shaw, a researcher based in the ICB's offices in São Paulo, also identified in patients with American tegumentary leishmaniasis what seems to a species not yet described in the scientific literature of the parasite that causes the disease. Until now, it is known that six species of protozoons of the *Leishmania* genus, transmitted to man by mosquitoes of the *Lutzomyia* genus, unleash in Brazil the skin infection in people: *Leishmania braziliensis*, *L. amazonensis*, *L. guyanensis* (the three most important), *L. lainsoni*, *L. naiffi and L. shawi*. "We have strong evidence that we have discovered a seventh species of the parasite that also causes tegumentary leishmaniasis", says Camargo. In his studies on the disease, the head of the ICB's Amazonian nucleus also enjoys the help of a doctor, Sérgio Basano, to whom he gave guidance for his master's degree at USP, and one of the members of the institute's team in Rondônia, which can use two laboratories and a small rural area for field research.

José Máximo, a fisherman from Vila Candelária: treatment for asymptomatic carriers of the Plasmodium may change the course of malaria

The scientist who traded the Seine for the Madeira

When he left behind a brilliant 32-year career on French soil and his last job at the Pasteur Institute in Paris, as head of the Experimental Parasitology unit, Luiz Hildebrando Pereira da Silva – or Professor Hildebrando, as he is usually called – started his much dreamt of returning to his native land, with the intention of giving priority to the study of the molecular, clinical and epidemiological aspects of complications arising from severe cases of malaria, the ones caused by *P. falciparum*.

In 1997, then nearing the age of 70, he speeded up his retirement from the Pasteur and sat for an examination to be appointed professor of the ICB/USP. He passed, and instead of working on one of the campuses located in the state of São Paulo, set himself up in Porto Velho. After all, at the beginning of the Nineties, Rondônia, with its 1.2 million inhabitants (less than 1% of the population of Brazil) had an attractive profile for working with malaria – it concentrated half the cases in the country, between 250,000 and 300,000 cases a year. One quarter of the cases in the three Americas.

Today, five years after having traded the banks of the Seine for those of the Madeira, and now retired from USP, though active with Cepem and with his studies of malaria partly financed by FAPESP, Luiz Hildebrando has not entirely succeeded in achieving his goal. This is because the cases of malaria from *P. falciparum* in Rondônia, around 20% of the total of cases in the state, have become scarce in Cepem's area of activity. Or rather, the complications arising from the severe cases have become rare.

In a universe of 1,500 cases of the illness, caused by the action of *P. falciparum*, that passed through the hands of Cepem's researchers over a period of two years, only two patients ended up showing severe complications as a result of the disease. The moral of the story: the simple presence of the teams from Cepem, eager to understand the peculiarities of Brazilian malaria – and well trained in diagnosing and treating the disorder, and intervening rapidly in the more severe cases that can lead to death or to cause permanent damages – has made the object of the parasitologist's study dwindle.

A confessed communist and a committed scientist, Luiz Hildebrando, born in Santos, São Paulo, but almost as French as he is Brazilian, is far from being a common scientist. To start with his age, 73. At this stage of life, people think more of retirement than of work, but Professor Hildebrando, who looks ten years younger, almost always does his stint at Cepem at the weekends.

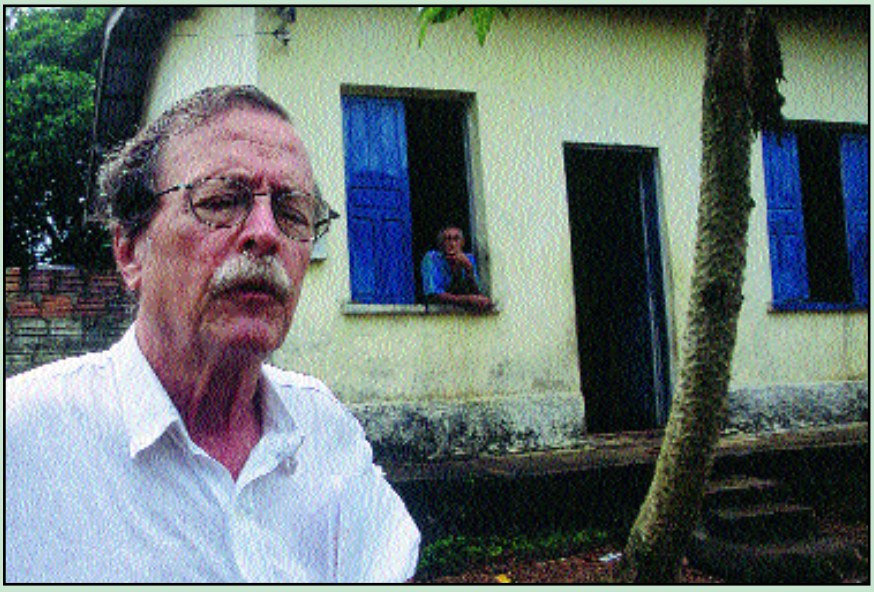
In reality, he is now officially retired. Three times, indeed. Once in France, from the Pasteur Institute, in 1997, after three decades of services rendered. And twice in Brazil, both times from USP: the first in 1980, through an administrative act that was a sort of reparation for having been twice turned away from the university during the military dictatorship, and the second in 1998, one year after having returned to USP, via the examination for incumbent professor, when he reached 70, the maximum age for a public servant.

Before anyone thinks that this great parasitologist is a maharajah (ironic definition used in Brazil for public servant who make a lot of money) of the civil service, it has to be said that the

Also in Monte Negro, the researchers found an extremely high occurence of an almost unknown skin disease caused by fungi found in animal remains and forest detritus; it is called chromoblastomycosis, and is rarely diagnosed, since it is often mistaken for American tegumentary leishmaniasis, which causes skin lesions. Amongst the inhabitants of the town, ten occurrences of the diseases, which also causes skin lesions, were identified between 1997 and 2001. "This gives an annual rate of incidence of 1.6 cases of the disease per 10,000 inhabitants, the highest in the world, and it suggests that chromoblastomycosis ought to be highly prevalent in the neighboring municipalities as well", says Camargo. The country that shows the highest level of chromoblastomycosis is Madagascar, with 1.2 cases per 10,000 inhabitants.

To study tropical diseases, scientists obviously have to have direct access to cases of these maladies. In Porto Velho, many sick people, when they feel a higher temperature, which may be a symptom of malaria, spontaneously seek the services of Cepem in Porto Velho. Last year, 18,000 patients were examined there, to see if they had this illness. And seven thousand of them did have malaria. The diagnosis for the other eleven thousand was inconclusive – laboratory tests were unable to establish the cause of the problem, an indication that there is a fertile field for anyone wanting to study new diseases in Rondônia.

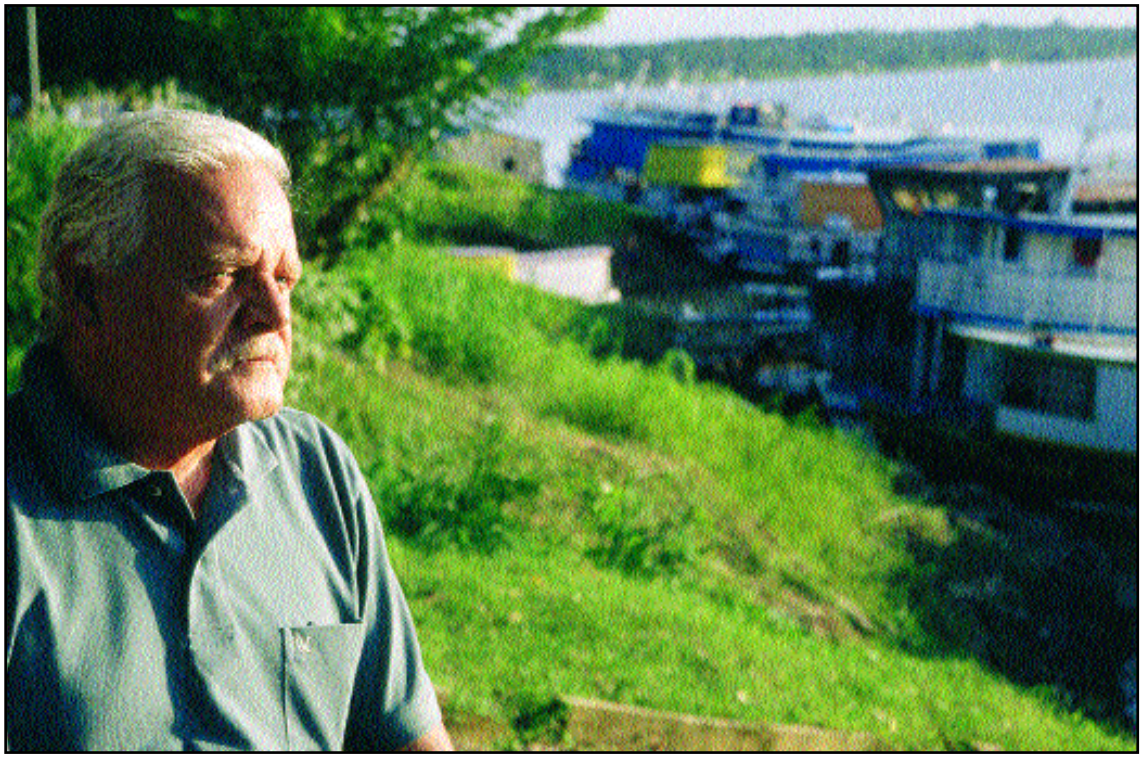
In little Monte Negro, the population has also got used now to the permanent presence of researchers from the ICB/USP and from other universities, who regularly provide medical assistance, helping the local authority in the task of taking care of its inhabitants. But it not always that the patients manage or are able to go to meet the researchers. The scholars from Cepem and the ICB then have to go regularly into the field, visiting areas where the access is more difficult. Even though on many trips, instead of finding some rare disease, the researchers do no more than practice good general medicine on the needy population – something rare in the poorer regions of Amazon region. Rondônia, for example, does not run any higher education course in medicine, and has one doctor for roughly every 2,200 inhabitants.



Luiz Hildebrando: admirable scientific rigor, social vision and fine sense of humor

last pension, the one for old age, earns him around R$ 30 a month. "But there is enough to lead a good life and to go to France once a year", says Luiz Hildebrando, who, for his work with Cepem, is rewarded with a grant for a visiting researcher from the National Council for Scientific and Technological Development (CNPq). Why go to France, if you have spent so much time next to the Eiffel Tower? Well, his wife and the majority of his children (and grandchildren) live there, and the scientist sees them for only three months of the year – the other nine months he spends in Porto Velho, studying malaria and other tropical diseases. "In future, I will gradually increase the months I spend in France, and reduce the time I stay in Brazil", says the researcher, who recently launched his second book of memories, *Crônicas de Nossa Época – Chronicles of Our Time* (Editora Paz e Terra).

If he does so, his colleagues at Cepem will miss him. They all admire him. Professor Hildebrando is the kind of researcher, with his undeniable technical competence and charisma for forming and leading groups, that is difficult to replace. Even though at times his scientific rigor may seem excessively European to Brazilians, a people that loves ways round things and ruses, and to Latinos in general. "If it is to be done the way Professor Hildebrando wants, following the scientific method, it is better not to do it at all" is the comment by Juan Abel Rodrigues, 26 years old, a Bolivian from Cochabamba, who is studying for his master's degree at Cepem, with a grant from Capes. "But this is what I most like about him." Oh, the professor also has a sense of humor, in his own way. At the end of January, on the day that followed the Brazilian football team's 6 – 0 win over Bolivia, in a game played in Goiânia, told Juan that Bolivia was the world champion in football. Football played at an altitude of 3,000 meters, of course. A reference to the fact that Juan's team only handles the ball well when the game is played in Bolivian towns at a high altitude, which leaves the visiting teams indisposed.



Plessmann on the banks of the Madeira river: "You never know when something different may appear, like a case of Ebola"

The World Health Organization (WHO) recommends at least one doctor for every thousand persons. São Paulo has one doctor for each 500 inhabitants. "The situation of malaria and of health in general would be much better in Rondônia just with an increase in the number of doctors in the state to something like one professional for every 1,000 or 1,500 inhabitants", says Luiz Hildebrando. That is why giving assistance to the rural communities, besides being a good source of input for scientific studies, is looked on as a moral duty by the researchers.

A day in the field may be more or less like this.

Around 8:30, Rui Durlacher, a 34 year old doctor from São Paulo, and Jussara Brito, a nurse from Rio Grande do Sul, of the same age, board the pick-up truck run by Cepem, which occupies a wing that used to belong to the Center for Tropical Medicine in Rondônia (Cemetron), one of the state-owned hospitals, on the outskirts of Porto Velho. At this time of day, there is already a movement of people with fever looking for a service that works only there, day and night, every day of the year: pricking a finger and taking a drop of blood, which indicates whether there is one more case of malaria, or dengue, or whatever else. But these are not the patients that lead the pair to settle into the automobile when the driver starts the engine. On Tuesdays and Thursdays, Durlacher and Jussara go to the municipality of Candeias, specifically to the

Genome that causes severe malaria deciphered

Six years of work and US$ 20 million were needed to decipher the genome of one of the four plasmodia that cause malaria. In February, English researchers from the Sanger Center and Americans from the Institute for Genomic Research (Tigr) concluded the sequencing of the genome of the *Plasmodium falciparum*, which causes the majority and the most severe cases of malaria in the world, 90% of which are concentrated in Africa (1 to 2 million deaths a year, above all of children). For a protozoon, of just one cell, *P. facilparum* shows a complex genetic code, more like the genome of an animal than of a bacterium. The 25 million base pairs of its DNA contain 5,600 genes, one sixth of the quantity found in man. The study of the functions of these bacteria and of the proteins produced by them may be vital for research into new drugs against the disease. In Brazil, there are three causal agents of the disease: *P. vivax* (80% of the cases), *P. malariae* (less than 1%) and *P. falciparum* itself (around 20%). In Africa, *P. falciparum* is transmitted to man mainly by the Anopheles *gambiae* mosquito, eradicated from Brazil, the genome of which is being sequenced by an international consortium of laboratories, amongst which the Onsa network (Organization for the Sequencing and Analysis of Nucleotides), set up by FAPESP.

Doctor Rui Durlacher, from São Paulo, examines 62 year old Perpétua Severiano, who has high blood pressure: assistance is the researchers' moral duty

rural district of Triunfo, with 2,700 residents, the majority with countless attacks of malaria, not to mention other health problems.

The journey lasts about an hour and a half, with quick stops at the modest health post in Candeias – there is no hospital in the center of the municipality, which has 13,000 inhabitants, including those from Triunfo – and at an office of Funasa by the roadside of the federal highway BR 364. The trip to the final destination covers 110 kilometers, 80 on the federal highway, which is even well kept in this stretch, and 30 on a dirt road, called a line, in the jargon of the region.

In the words of 42-year old Antonio Eusébio da Silva, four times a victim of malaria, who comes from Minas Gerais and now administers the urban area of Triunfo: "The name for health in Triunfo is Cepem and Doctor Rui. If they leave here, we are in trouble". Durlacher explains that the district – in spite of being home to less than one quarter of the population of Candeias, – accounted for almost half the cases of malaria of the municipality a few years ago. Today, there are inhabitants who have not caught the disease for over two years.

At the nucleus, Durlacher and Jussara pass by the local health post. They talk to the staff, take care of patients, and get back on board the pick-up en route for the lesser lines of Triunfo, the narrowest and most pot-holed local road that lead to the most far-flung communities. On a productive day, they visit at least six houses, to follow up the progress of the various clinical conditions. They look after any health problem that arises: high blood pressure in the aged, undernourishment and diarrhea in children, the evolution of a pregnancy, a baby that has been too long in the sun and got burnt. "We do not chose the patient", comments Jussara, in the best general practice.

At dusk, now time to go back to base, it is not uncommon for the researchers to come across a scene similar to the photograph that begins this article: children and adults refreshing themselves innocently in a creek or lagoon. Precisely at the time, the end of the day, and in the place, on the riverside, that the mosquito that transmits malaria in Amazon region, *Anopheles darlingi*, likes to bite its victims. It is probable that red cloths will shortly be hung outside the houses of the formerly happy bathers, as a sign used by the inhabitants to advise the motor bike riders from the town hall, who pass by there periodically, that there are folks with a high temperature and under suspicion of malaria in that house, in need of a blood test urgently. •

THE PROJECTS

Survey of the tick fauna of Rondônia and Determining the Prevalence of Rickettsia, Erlichia and Borrelia in these arthropods

MODALITY
Thematic project

COORDINATOR
Erney Felício Plessmann Camargo – ICB/USP

INVESTMENT
R$ 410,079.07

Variant antigens of Plasmodium Falciparum: Participation of the Phenomenon of Cytoadherence and Repercussions in the Pathogeny of severe Malaria

MODALITY
Regular research benefit line

COORDINATOR
Luiz Hildebrando Pereira da Silva – ICB/USP

INVESTMENT
R$ 392,269.81