Reversing the course of malária

Scientific research, control of endemic areas and coordinated action by institutions are reducing the number of malaria cases in Rondônia

Claudia Izique

The incidence of malaria in Rondônia has fallen sharply over the last two decades and at a faster pace than in the rest of the Amazon region. But in the city of Porto Velho, the state capital, the number of cases has remained steady and even increased slightly in recent years. How do we explain this contradiction? It should be noted that in the Amazon region as a whole the number of cases declined from 600,000 in 1999 to fewer than 300,000 in 2011. In Rondônia, during the 1980s and 1990s, the number represented 40% of all cases reported in the Amazon and today it represents 12%. Credit for this result must be given to control measures that have improved markedly in recent years. For example, new drugs such as Artesunate have been introduced, and health authorities have provided scientific and technical advice. Credit must also be given to the efforts of the Center for Research in Tropical Medicine (Cepem) at Rondônia's State Department of Health, an advanced center of the Institute of Biomedical Sciences (ICB) at the University of São Paulo (USP), the Tropical Pathology Research Institute in Rondônia (Ipepatro), and the stubborn nature of Luiz Hildebrando Pereira da Silva.

FAPESP participated in this effort to combat malaria by supporting the research projects of Hildebrando and two ICB-USP researchers Erney Plessmann de Camargo and Fabiana Piovesan Alves. From October 1999 to December 2002, FAPESP developed a study of the presence of asymptomatic *vivax* malaria in hypoendemic areas of the Amazon region as a doctoral project, directed by USP professor Camargo. He expanded his research into neglected tropical diseases to survey the tick fauna of Rondônia and determine the prevalence of microorganisms *Rickettsia, Erlichia* and *Borrelia* in these arthropods, which can cause infections in humans. Camargo's project, which ran from 2000 to 2004, also involved diagnosing these infections and establishing a health surveillance system in Monte Negro, Rondônia. As to Hildebrando's work, from 1999 to 2001, his FAPESP-supported project in this area involved the study of variant antigens of *Plasmodium falciparum* and their relationship to severe forms of malaria. In values at the time, the two largest research grants for PhD scholarship awarded by the Foundation for these studies amounted to an investment of R$876 million.

Luiz Hildebrando has been studying tropical diseases since the 1950s. He has published 80 scientific articles on malaria and was responsible for the establishment of Cepem and Ipepatro. As professor of parasitology at the USP School of Medicine he was exiled to France, where, over the course of 32 years, he directed the units of cellular differentiation and experimental parasitology of the Pasteur Institute and investigated the mo-

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lecular biology of the malaria parasite in French Guyana, Madagascar and Senegal. He retired in 1997 and returned to Brazil the following year to settle in Rondônia, where today at age 84 he studies the immunology and epidemiology of the disease.

Pesquisa FAPESP has interviewed him twice over the past ten years: first in 2002, when reporter Marcos Pivetta visited him in Porto Velho, and then in 2007 in São Paulo. This time, however, Hildebrando saved the reporter legwork by responding in writing to questions to be used in a story to update the progress of malaria research. He told us that results obtained in recent studies by teams in Rondônia using innovative control methods make him think that eradication of malaria in the Amazon region is a very real possibility within the next decade. Luiz Hildebrando, author of the recently published book *Subversive Chronicles of a Scientist,* is an absolute master of his stories.

> "Mobility of the Amazonian populations is the critical factor in maintaining and spreading malaria"

In March 2002, you told us that Cepem had determined that victims of P. vivax are able to transmit the disease even though they show no symptoms of malaria. Has this been confirmed?

This work was published in the British journal *Lancet* in 1999, and referred to asymptomatic carriers of *vivax* malaria. Studies have advanced considerably in recent years. Unfortunately, in 1997, USP decided to move the Institute of Biomedical Sciences from Porto Velho to the interior of Rondônia State. At the same time, the Tropical Pathology Research Institute (Ipepatro), which obtained funding from the Ministry of Science, Technology and Innovation (MCTI), was established in Porto Velho to conduct research on malaria along with Cepem. The collaboration continued with researchers from the Department of Parasitology and with several other USP researchers in the cities of São Paulo and Ribeirão Preto and with the federal universities of Rio de Janeiro (UFRJ) and Mato Grosso (UFMT). In this new phase, they were able to discover the presence of asymptomatic carriers of the *vivax* malaria and *falciparum* malaria parasites in endemic areas of Rondônia, particularly in riverine areas. The 2005 research resulted in 16 publications, 3 doctoral theses and 12 master's degrees in the graduate program in experimental biology administered by the Federal University of Rondônia. It demonstrated the importance of mobility on the part of the Amazonian populations in maintaining and spreading malaria in the endemic areas. In addition to showing that asymptomatic carriers of parasites are sources of infection in maintaining endemic disease, it demonstrated the role that relapses of *vivax* malaria have, even after a full course of treatment with Primaquine. Analysis of the role of different Anopheles species in transmission was conducted, and a conclusion regarding the dominant role played almost exclusively by *Anopheles darlingi* in endemic transmission was

reached. The study also described the cycles of seasonal variation in their vector densities.

What is the research agenda today?

In recent years, we have concentrated on the study of two factors related to the process that maintains the high incidence of malaria in the region: 1) the role of asymptomatic carriers of parasites, in both *falciparum* malaria and *vivax* malaria; and 2) the problem of relapses in *vivax* malaria. The past five years has seen a significant drop in the total number of malaria cases, particularly *falciparum* malaria. Until the late 1990s, it had represented about 20 – 25% of the total number of cases, but now represents only 10 – 15%. This success came about thanks to the efforts of the Ministry of Health and the introduction of a treatment by two drugs that are derivatives of Artesunate. From this trend, our research has shown the positive effect of supplementary treatment of asymptomatic patients with *Plasmodium falciparum* in a pilot project in locations where the transmission of *falciparum* malaria was zero for about a year and reintroduced only by external contamination. The same level of success was not obtained with *vivax* malaria however. On the contrary: there was no significant variation in post-treatment incidence of asymptomatic carriers essentially because clinical (symptomatic) relapses were observed even among asymptomatic carriers who had been treated. This result led us to the original finding that clinical immunity in *vivax* malaria is a specific variant and not a specific species, due essentially to the polyclonal nature of the hypnozoite population in liver cells (latent forms of p. vivax in the livers of infected patients). Finally, in recent years, a new procedure was able to be developed to prevent relapses. Traditionally, patients with symptomatic and asymptomatic clinical forms would undergo a short period of preventive treatment of relapses by Chloroquine, which acts not on hypnozoites in the liver, but on asexual blood forms from the liver in the first infection or in relapses. With this new procedure, inspired by actions taken in New Guinea and in sub-Saharan Africa known as IPT (Intermittent Preventive Treatment), we made an adaptation for the conditions found in the Amazon region that we called SIPT (Selective Interrupted Preventive Treatment). We obtained great success, reducing malaria transmission to extremely low levels in pilot riverine locations. We are now trying to reproduce this procedure more extensively and, at the same time, in more locations. It is important to point out that the operation's success depends on the proper functioning of the Family Health Program (PSF) infrastructure in the localities in which it is applied.

What is the current rate of asymptomatic malaria?

In the riverine areas of the Madeira and Machado rivers that were studied, the current rates range between 10 – 40% of adult residents.

What areas are being studied?

In previous work we showed that the incidence of malaria is particularly high in riverine areas, where the Anophelic population in the rainy season rises to high levels of HBR (Human Biting Rate) density, over 20 or 30 (bites per person per hour). The development of natural clinical immunity, responsible for being considered an asymptomatic carrier, is directly related to the frequency of exposure to infectious bites and, therefore, to the amount of time in residence in the transmission areas. We therefore performed studies on asymptomatic carriers of malaria parasites primarily in the riverine areas of Rondônia. These areas are situated throughout the city of Porto Velho (35,000 square kilometers), which occupies a patch of land that is approximately 500 km long and 60 km wide along the Madeira River, stretching from Rondônia's border with Mato Grosso to its border with the state of Amazonas.

Progressive victory against malaria

Year	Total Tests	Total (%) Positive
TESTS PERFORMED AT CEPEM		
2006	26,518	9,324 (35.2)
2007	26,956	7,671 (28.5)
2008	18,465	4,038 (21.9)
2009	18.319	3,299 (18)
2010	17,001	3,627 (21.3)
2011	13,202	3,047 (23.1)
PORTO VELHO		
2006	142,188	34,865 (24.5)
2007	137,529	32,932 (23.9)
2008	119,635	23,647 (19.8)
2009	141,469	20,591 (14.6)
2010	152,191	23,257 (15.3)
2011	122,531	19,266 (15.7)
RONDÔNIA		
2006	407,997	101,646 (24.9)
2007	372,167	81,929 (22.0)
2008	269,364	49,807 (18.5)
2009	277,289	41,366 (14.9)
2010	262,070	43,576 (16.6)
2011	212,146	35,120 (16.6)
BRAZIL		
2006	2,959,134	549,379 (18.6)
2007	2,983,535	457,433 (15.3)
2008	2,724,433	314,879 (11.6)
2009	2,618,715	308,407 (11.8)
2010	2,710,800	333,404 (12.3)
2011	2,562,497	293,520 (11.5)

TABLE SOURCE DATA FROM SIVEP MALARIA OF THE MINISTRY OF HEALTH

How many tests does Cepem perform annually?
Tests for malaria are performed by our team of microscopists stationed in Cepem's center for the diagnosis and treatment of malaria, located in an annex to Cemetron Hospital, a part of the State Department of Health (Sesau). It specializes in infectious and parasitic diseases and pathologies and is open 24 hours a day 365 days a year, including Sundays and holidays. Its services and assistance to the population are also useful for research into the pathophysiology, immunopathology and chemotherapy of malaria developed in direct collaboration and with the financing of Ipepatro/Fiocruz-Rondônia (Oswaldo Cruz Foundation). In this case, patient volunteers are selected to participate in various research programs, attended by doctors and technicians who have equipment for biochemical, hematological, serologic, immunological and molecular tests such as FACS (fluorescence-activated cell sorting), fluorescent microscopy, equipment for ELISA (enzyme-linked immunosorbent assay), PCR (polymerase chain reaction) and real-time PCR analysis. Cepem has a special team for ethical control of studies involving humans.

In recent years we have experienced a 50% reduction in the number of malaria cases seen at Cepem and a more than 85% reduction in the number of cases of *falciparum* malaria. This reflects the general trend of malaria in the entire Amazon region, in Rondônia and specifically in Porto Velho. The number of cases in Rondônia, which in the 1980s and 1990s represented more than 40% of the total number of cases in the Amazon, came to represent only 18.5% in 2006, falling even more in 2011, to 12%. However, data from SIVEP-Malaria shows a relative increase in the incidence of malaria in Porto Velho, which accounted for 34.3% of the total number of cases in the state of Rondônia in 2006, progressively rising in 2009, 2010 and 2011 to represent 49.8%, 53.4% and 54.9%, respectively, of the total number of cases in the state. This situation is due in large part to the movement of populations caused in recent years by the Madeira River hydroelectric work.

In addition to the tests at Cepem, fieldwork tests are conducted primarily in riverine areas of the Madeira River for purposes of epidemiological and control studies. Teams of microscopists are stationed in the areas of Vila Amazonas, Cachoeira de Teotônio and São Sebastião. Blood samples are transported by a fleet of vehicles to Cepem for processing. The vehicles are also used for fieldwork in entomology, an area that experienced significant expansion after Ipepatro joined Fiocruz.

> "In recent years, we have seen a 50% reduction in the number of malaria cases treated at Cepem"

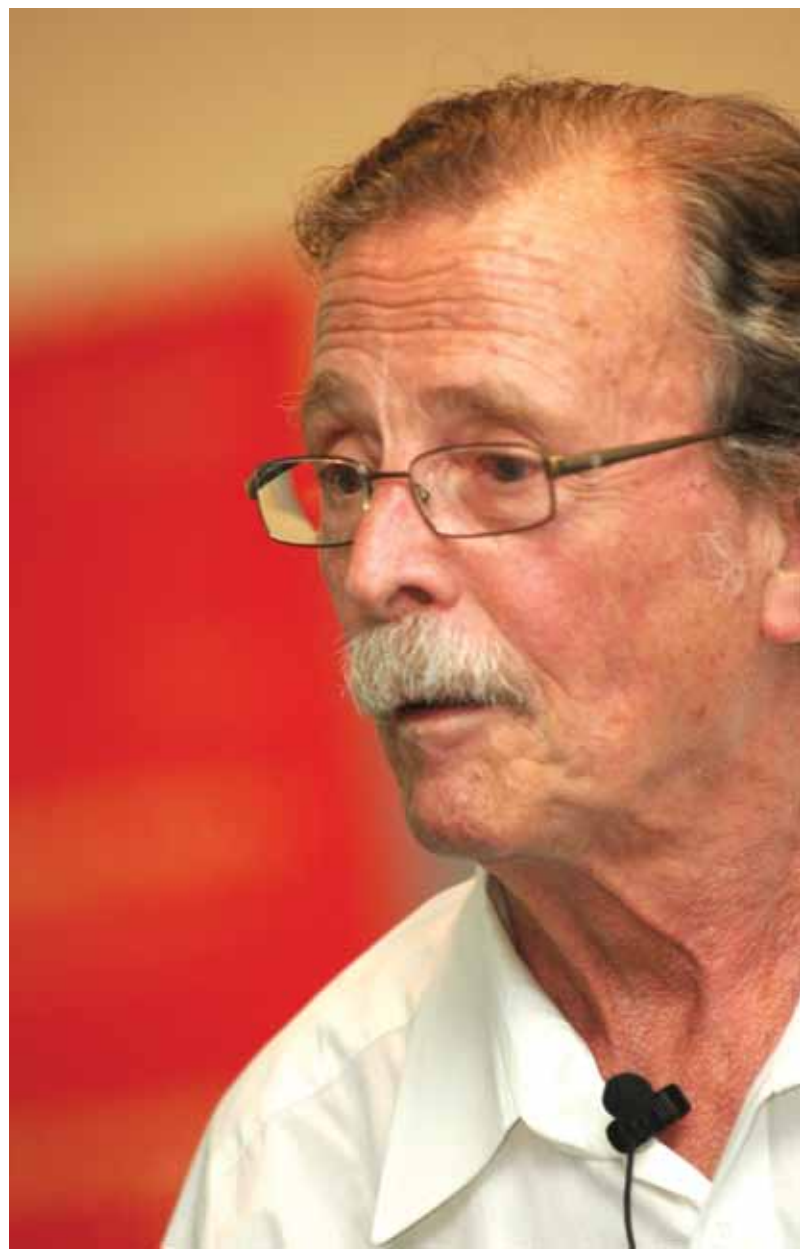
In 2007, you were concerned about the effect migration was having on the expansion of the disease. Were you right to worry?
In 2009, we published an article in *PLoS ONE* about the dynamics of transmission and spatial distribution of malaria in riverine areas of Porto Velho. We reached the following important conclusions: 1) even though transmission may occur intra- and extra-domicile, maintaining malaria in endemic areas essentially depends on intra-domicile transmission for its spread among residents of the endemic foci, reinforced by the presence of permanent sources of infection (asymptomatic carriers); 2) the mobility of Amazonian populations is the critical factor in spreading and extending endemic malaria. In the case of two riverine areas, visitors and temporary residents (seasonal fishermen) were responsible for an increase of 2.6 times in the number of reported cases. In 2007 there were 102 cases of malaria among the 379 residents and 265 cases among visitors and temporary residents (fishermen), particularly during the spawning months, when fish go upriver to spawn and are more easily caught. However, these fishermen were residents of the outskirts of Porto Velho and carriers of malaria to the outlying areas of the city. The results put us in a position to refute the approach by the World Health Organization's Global Malaria Program that was based on the world map drawn up by teams at the University of Oxford. Considering the "low incidence of *falciparum* malaria in the Amazon region," it proposed the use of traditional means for control, specifically the use of impregnated bed nets associated with the treatment of malaria. We advocate a different approach to control, to instead identify foci of high endemic risk in order to concentrate the instruments of control on both the vectors and

the domiciles, such as by treating permanent residents of the foci, including asymptomatic carriers of parasites.

Has the incidence of the disease increased with the construction of the Santo Antônio hydroelectric power plant on the Madeira River, and the resulting migration?

In fact, the content of the *PLoS ONE* article was the result of studies and surveys we conducted from 2006 to 2007, which took into account the construction work associated with the Santo Antônio hydroelectric power plant. We mapped the riverine areas throughout the hydroelectric impact zone, identifying residences and residents, and we planned the installation of surveillance and control units that we thought were needed to control operations in the area. In mid 2007, we prepared a long document that contained ten proposals to prevent the potential worsening and spread of malaria in the riverine region of Porto Velho and its surrounding areas. Convinced that the danger came from existing high endemic foci in the riverine areas of the hydroelectric impact zone, the document proposed setting up five treatment and surveillance units throughout the Madeira riverine area with Family Health Program (PSF) teams. It also included a unit called the Operational Coordination Center, equipped with mobile units, a training center, an entomology laboratory for vectors, and operational annexes. The proposal suggested establishing a process to authorize a multi-institutional mechanism for technical cooperation and shared responsibility, bringing state and municipal health services together with construction companies, to form a purely technical health supervision sector in the hydroelectric impact zone. Unfortunately, the proposal did not go forward because each sector had competing political interests or financial priorities. This led to a fragmentation of authority and mechanisms of action. The hydroelectric power consortium ended up with authority over the construction site. The municipality as compensation requested funds to deploy basic health services, both within and outside of the impact zone. The state, with the same arguments, requested funds for hospital construction outside the impact zone. The Ministry was designated responsible for "supervising" the oversight activities, which became difficult to do because the actors and responsible parties were so dispersed. Not to mention the extraterritorial right of the actors, granted to the private companies (hydroelectric companies) in making decisions in the area of health, in addition to what they would do with their employees (which is legitimate), to remain free to use vector control measures in residential areas of the local population that had not been adopted by the Ministry of Health's vector control. Because authority was dispersed, no one ended up responsible for sanitation, which should have been a priority for vector control in the impacted urban, suburban and neighboring rural areas. It would have permitted the control of malaria in this area as well as control of waterborne diseases such as infantile diarrhea, typhoid fever, amoebiasis, leptospirosis, hepatitis A, and other diseases endemic in the area.

This lack of coordination among federal, state and municipal health authorities, as well as their interaction with the private sector, which should be thoroughly coordinated according to the National Health Care System (SUS) policy, continues to lead to mistakes. When we received funds to control malaria from the WHO Global Fund in 2010, we were required by the donor – the Fund – to reserve US$ 20 million of the aide to purchase



MARCIA MINILLO

and deploy mosquito nets impregnated with insecticides in the control campaigns. However, the use of insecticide-impregnated nets, so valued by the WHO Global Fund, has not been proven effective against the type of malaria we have in the Amazon region. Aside from this, receiving financial aide already designated for a specific type of measure to be financed and used, runs counter to the most basic principles of administrative autonomy of the Republic. More serious still is the fact that the WHO requires the funds to be managed not by the Ministry of Health, but by an established and accredited private entity. Interestingly, after transferring the first installment of the aide, WHO discovered that Brazil ranked sixth in the world in GDP and thus should not have received aide. WHO reneged on the agreement and did not transfer the second installment. However, the department of the Ministry of Health that was responsible had already organized the distribution of impregnated bed nets and now intends to obtain public funds (including from the Science, Technology and Innovation (CT&I) sectoral fund of the Ministry of Health) to determine whether the many thousands of insecticide-impregnated nets already purchased, distributed and set up would even be useful for something in relation to malaria, since, on the other hand, the widespread use of electric lights attracts all manner of insects. No one actually knows what impact this equipment will have on the insect fauna of the Amazon region. The impregnated mosquito nets are being set up without having conducted impact studies, within the greatest biodiversity on the planet. Just try to sleep with that kind of noise.

In 2007, you said you were about to register a patent for a drug-based chemotherapy developed from biodiversity. Has the patent been filed?
There are two lines of research. The first involves the production of antibodies from camelids and the second seeks funding to identify prototypes/products for the prevention and treatment of neglected diseases, based on Brazilian biodiversity primarily from the Amazon region. In the latter case, we are concentrating our efforts on malaria, leishmaniasis and Chagas disease. Two patent applications from our technology management center have been accepted, but further details on the drug's toxicity are required for permanent registration. There are two promising active substances against *Leishmania sp*, extracted from regional plants that were shown to be active against epimastigote forms as well as intracellular amastigote forms. Research shows that these substances inhibit enzymes from the biosynthetic pathway of the parasite's nucleic acids. These substances are undergoing nanoassembly on nanostructures for controlled dispersion and the results are very promising, especially for skin lesions. In the near future, we will have the pleasure of announcing them. Regarding the study of camelid antibodies, there have been promising results in neutralizing rabies and the local effects of poisoning by animal toxins. The start-up of the Center for Applied Biomolecular Studies in Medicine (Cebio), under the direction of Rodrigo Stabeli, was a significant development for research into new active compounds, which are obtained from biodiversity to combat neglected disease agents. Created with funds obtained initially from Ipepatro and Finep, it was established in collaboration with the Federal University of Rondônia. Since Ipepatro became Fiocruz-Rondônia, this line of research has become strategic. The pool of biodiversity research is coordinated and seeks to isolate and develop drug prototypes and immunological materials active against malaria, leishmaniasis, Chagas disease, rabies and other neglected diseases such as those produced by arboviruses and animal toxins. Cebio has developed a number of new technologies, which use nanosensors, mass spectrometry and two-dimensional electrophoresis against defined molecular targets in order to isolate and identify bioactive natural products or immunobiologicals. We now produce recombinant enzymes by genetic engineering for three key enzymes as molecular targets of *Plasmodium falciparum* in order to isolate active natural products. We produce recombinant enzymes of *Leishmania amazonensis* for the same purpose. Cebio is the only center that uses nanosensors to produce results from trimeric enzymes in Biacore (data still under patent protection). Using the Phage display technique, we have produced monomeric type VHH antibodies isolated from camelids (llamas and alpacas) against the yellow fever virus and rabies as well as snake toxins. The

"We have produced antibodies isolated from llamas and alpacas against the yellow fever virus and rabies as well as snake toxins."

In Candeias, Rondônia, the red cloth is a sign that there is a suspected case of malaria.

EDUARDO CESAR

antibodies research focuses on the ability of the isolated VHH antibodies to inactivate viruses and toxins in vivo.

What is the market outlook for these results? I answer this question by citing the example of Spirodolona, which is one of the only new antimalarial drug that the international pharmaceutical industry is proposing for entry into the clinical trials phase. If all goes well, we anticipate a minimum of three to four years for it to reach the market. An article in the December 2011 issue of *Science* describes the process in which 29 signatories collaborated, among them a number of malaria experts from 12 respected Swiss, English, American, Thai and Singaporean institutions, in addition to researchers from Novartis. To produce Spirodolona, we started with 12,000 natural and synthetic products with indications of antimalarial activity. In the first round we selected 275 of them that had less than 50% toxicity against human cells at a concentration of 10uM and antimalarial activity at a concentration of less than 1uM. We then isolated the 17 most active of the 275 products, from which 200 analogs were synthesized, with minor structural variations and tested *in vitro* and *in vivo* to arrive at Spirodolona, which selectively inhibits protein synthesis of *Plasmodium falciparum*. Several recent publications have confirmed that in cutting-edge pharmaceutical companies it takes a new product an average of 13.5 years to go from its first discovery to a marketing plan. It also requires dozens of technicians and specialists for a succession of selective studies, consumes hundreds (or thousands) of laboratory animals and requires the use of the latest generation of extremely expensive equipment.

This digression was intended to highlight the difficulties Brazilian scientific and academic institutions face in developing similar studies. They have to rely solely on students and interns who are preparing undergraduate or graduate papers, to whom project leaders cannot give routine activities as a topic to select at various stages, for example, repetitive manipulations of surviving molecules from progressive screenings. Because our pharmaceutical industry does not have sufficient resources to invest in "promising" research, and development institutions generally only finance research training for undergraduate, master's and doctor's degrees, this problem must be solved by developing research fellows and permanent technical support positions. Without this, our discoveries of promising products will always be at the initial level of description and, obviously, we will be the ones to suffer the consequences as materials related to local diseases become increasingly scarce, until the outbreak of this or that now neglected disease starts to turn a profit. We cannot just follow or become hostages to the local diagnostic pharmaceutical market. Brazil has great minds connected to the development of public health inputs. We need a strategic public policy to coordinate development of this area. ■

PROJECTS

1. *Variant antigens of Plasmodium falciparum: participation in the phenomenon of cytoadherence and implications in the pathogenesis of severe malaria* – No. 1998/12107-2 (1999-2001)
2. *Survey of the tick fauna of Rondônia and determination of the prevalence of Rickettsia, Erlichia and Borrelia in these arthropods* – No. 1999/08589-4 (2000-2004)
3. *Study of asymptomatic vivax malaria in hypoendemic areas of the Brazilian Amazon* – No. 1999/06603-0 (1999-2002)

GRANT MECHANISMS
1. Research assistance – regular
2. Thematic project
3. PhD scholarship

COORDINATORS
1. Luiz Hildebrando Pereira da Silva – ICB/USP
2. Erney Felicio Plessmann de Camargo – ICB/USP
3. Fabiana Piovesan Alves – ICB/USP

INVESTMENTS
1. R$392,269.81
2. R$398,242.50
3. R$85,459.53

SCIENTIFIC ARTICLES

ALVES, F.P. et al. Asymptomatic carriers of Plasmodium spp. as infection source for malaria vector mosquitoes in the Brazilian Amazon. **Journal of Medical Entomology.** v. 42, p. 777-9, 2005.

LABRUNA, M.B. et al. Rickettsia belli and Rickettsia amblyommii in Amblyoma ticks from the state of Rondônia. **Journal of Medical Entomology.** v. 41, p. 1073-81, 2004.

GBOTOSHO, G. O. et al. Different patterns of pfcrt and pfmdr1 polymorphisms in *P. falciparum* isolates from Nigeria and Brazil: the potential role of antimalarial drug selection pressure. **American Journal of Tropical Medicine and Hygiene**. v. 86, p. 211- 13, 2012.

FROM OUR ARCHIVES

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