

GENOMICS

On the trail of a vaccine

Brazilians sequence the genome of the bacterium that causes leptospirosis and request patents for twenty-four proteins

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Anyone who has read the issue of the 24th of April of the British magazine *Nature*, one of the most influential scientific publications, verified that a team from the Chinese Human Genome Center of Shanghai, had sequenced the complete genome of the Lai strain of *Leptospira interrogans*, the most common of the bacteria that causes leptospirosis in that country. Transmitted to man by the urine of rats and other animals infected with the pathogen, leptospirosis is considered the most widespread of the zoonoses, the diseases that animals transmit to human beings, especially in rural areas with a humid climate. Carried out by scientists from a developing nation outside of the axis Europe – United States, the work of the Asian group was, without a doubt, of international relevance. But for the Brazilians the most important news about



PHOTOGRAPH BY EDUARDO CESAR

Open-air sewer:
contact with contaminated
water boosts the
risk of transmission
of leptospirosis

leptospirosis does not come from the Far East nor does it arrive via the pages of some publication – and has been, until now kept in silence. Working with discretion on a project in competition to the Chinese work, a research team from the Butantan Institute of Sao Paulo, with collaboration from colleagues at the branch of the Osvaldo Cruz Foundation (Fiocruz) in the state of Bahia and with São Paulo universities, have also concluded the complete sequencing of another lineage of *L. interrogans*, known as serovar Copenhageni, responsible for the majority of human cases of the diseases in Brazil.

Integrated research - In reality, the Brazilian group did more than simply unmask the molecular structure of a pathogen similar to that mapped by the scientists from Shanghai. They also took a sure step towards strengthening a promising line of research with the goal of developing better forms of prophylaxis and diagnosis of human leptospirosis, the project's main goal. This is because in February 2002, one year and two months before the Chinese had published their article in *Nature*, the Butantan scientists had requested, in the United States, the patents for twenty four genes – and their respective proteins – identified during the work on sequencing and analysis of the genome of serovar Copenhageni, carried out by the AEG, the public network of São Paulo laboratories that specializes in genomes concerning agronomy and the environment. “These proteins could be useful for the development of a vaccine against human leptospirosis (today there is none) or for more efficient tests for the diagnosis of different serological forms of the illness”, says Ana Lucia Tabet Oller do Nascimento, from Butantan, the coordinator of the project on *L. interrogans*, which was funded by FAPESP. Such a hypothesis is based on preliminary tests in the laboratory that have shown that this group of twenty four proteins react on contact with the blood serum of people or rats infected

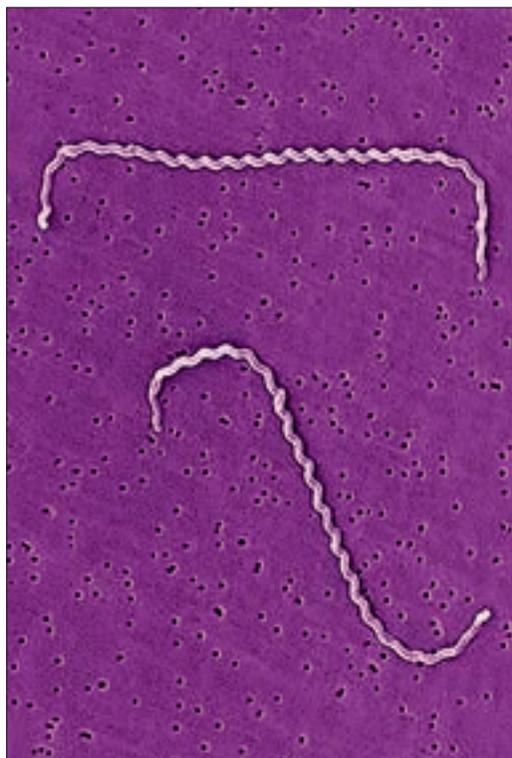


IMAGE BY CDC/NCID/HIP/JUANIE CARR

Leptospira interrogans: transmitted mainly by the urine of rats

with leptospirosis. The reply to the patent request, whose rights extend to Brazil, should come out next year.

Comparative study - The Butantan researchers have just completed a scientific paper comparing the genomes of the two varieties of *L. interrogans*. Much of the information on the work, which was submitted to a large international magazine and is awaiting the green light for publication, is still confidential. However, some general data, results of this comparison, can already be released. The two genetic sequences show

themselves to be almost the same size. The serovar Lai has almost 4.7 million pairs of bases (the chemical units that make up the genetic code), divided in two circular chromosomes, one large and the other small. The serovar Copenhageni shows an almost analogous structure, also with two chromosomes, only that they represent around 60,000 fewer pairs of bases than the strain studied by the Asian group.

The greatest difference between the two strains is regarding to their probable number of genes and respective proteins. In their study, the Chinese counted some 4,727 genes and a similar number of proteins in the serovar Lai. Working with the serovar Copenhageni, the Brazilians counted around 3,700 genes or proteins. This is a considerable difference for two genomes apparently so close to each other. “We do not believe that there are so many

more proteins in the Lai in relation to the Copenhageni”, declares the biochemist Elizabeth Angélica Leme Martins, from the Butantan Institute, and an assistant on the project. “By our analysis, this number difference should be a lot lower, around 200 proteins.” Another contribution from the Butantan team, which also was able to count upon the participation of Paulo Lee Ho and Luciana Leite, was the discovery of 250 new proteins on the surface of serovar Copenhageni. These proteins stay on the membrane of the cells of the pathogen and are in direct contact with the host, man or animal infected by *L. interrogans*. Among this group of proteins, a smaller group of 174 lipoproteins stand out, these could have greater involvement in the process of infection caused by the bacterium. “Before our work began, only ten lipoproteins had been identified on *L. interrogans*”, says Ana Lucia. “The Chinese described only a few proteins of this type.”

In the towns - Differently from serovar Lai, typical of the flooded rice plantations of Asia, the platform for a large part of the occurrence of the illness

THE PROJECT

Sequencing of the Genome of Leptospira interrogans

MODALITY

Regular line of research assistance

COORDINATOR

ANA LUCIA TABET OLLER DO NASCIMENTO – Butantan Institute

INVESTMENT

R\$ 776,526.85

in China, the Copenhagen strain is the one that is principally responsible for the incidences of human leptospirosis in Brazil, where there have been a little more than 46,000 confirmed cases between 1987 and 2001, with an annual death rate of from 6.5% to 20% of those infected. Here, instead of being a problem associated with open fields, the illness has a more metropolitan character. In towns people usually catch the disease in areas without basic sanitation, with accumulated garbage (which attracts rats) and with open sewage, or during the time of the summer floods when the public roads transform themselves into dirty streams and increase the risk of contact with water or earth contaminated with the urine of rats infected by the bacterium. The sewage rat (*Rattus norvegicus*), an inhabitant almost as urban as modern man himself, is the main host for the serovar Copenhageni of *L. interrogans*.

Once infected, a person can take from two to thirty days to develop the most common symptoms coming from the presence of the bacterium in his organism: fever, headache, shivers, vomiting, nausea and general ill-feeling. If it is not treated with antibiotics, normally penicillin or doxycycline, the *L. interrogans* can affect the kidneys and liver, and in extreme cases, lead to death. As its clinical manifestations don't differ much from the symptoms unchained by other known illnesses such as dengue fever, and yellow fever, leptospirosis is often mistaken for other illnesses. In order to reach at a diagnosis with the minimum degree of confidence, it is necessary to carry out laboratory tests using blood or urine samples from the suspected case. "At times, even with these examinations, one will not manage to say exactly which serovar is responsible for the infection", Ana Lucia comments.

It may well seem as just a detail to determine the serovar of *L. interrogans* responsible for an infection, but this impression is false. To know the variety of the bacterium that unchains the di-

**Brazil and China
are studying
different
varieties
of the same
bacterium**

sease in a person helps to prevent the evolution of the infection, since there are strains with varying degrees of aggression, and it is of fundamental importance for the creation of a vaccine against this disease. This is even truer in the case of this pathogen.

There are more than 250 known serovars of *L. interrogans*, with varying degrees of common characteristics. To differentiate one type from another is not always easy from the point of view of its morphology, of its external appearance. Seen with the help of an electronic microscope, the varieties can be considered identical. All of them are fine and elongated, with a spiral form, without rigid cellular walls. "It is not possible to distinguish the varieties only by morphology", Elizabeth explains.

The problem is that, if its physical constitution does not change according to the serovar, other parameters are a long way from being always the same or similar in the diverse forms of the bacterium. The degree of pathogenicity, location of geographic occurrence, most common host and preferential victims (man or other animal such as a dog, cow or rat) can vary considerably in function of the strain of *L. interrogans*. For example, certain forms of the bacterium cause leptospirosis only in men, others infect only animals and there also exist others that attack both. The Copenhageni and the Lai are among the most virulent varieties of *L. interrogans* that attack the human being. In the study of the comparison of the genomes of these two strains, the Butantan researchers believe that they identified two proteins present only in the variety sequenced in Brazil – and involved in the synthesis of polysaccharides, a type of sugar – that could facilitate the process of differentiating between the serovars. "If confirmed, this piece of information could also be important for the understanding of the large antigenic variation of the *Leptospirae*", forecast Ana Lucia.

Brazilian variation - The main clinical implication of the large diversity of the pathogens responsible for leptos-

pirosis is the existence of multiple antigens associated with the disease, each one of them literally different from the others. Any substance recognized by the organism as of external origin or capable of offering danger to it is called an antigen. To defend against this potential aggressive agent, normally one or more proteins, the immunological system produces specific defenses (antibodies) against the antigen that is threatening it. In the case of leptospirosis, due to the high number of serovars of the bacteria that causes the illness, to have antibodies against a variety of *L. interrogans* does not necessarily confer protection against the other forms of the pathogen.

In an ideal scenario, the perfect vaccine against leptospirosis would confer immunological protection against all of the forms of the bacterium, or at least those most disseminated. Nevertheless, the maximum that can be obtained in many cases is a more focused and specific product, capable of impeding the infection brought on by one or another type of serovars and that shows itself innocuous to the other forms of the pathogen. The countries that are deeply researching into leptospirosis, which are few, tend to focus their work on the most important local varieties.

Besides China and Brazil, that have sequenced distinct varieties of *L. interrogans*, the Australians are studying the genetic material of another strain of this bacterium. "We have to carry out our very own research with the serovar Copenhageni, which is the major cause of human leptospirosis in Brazil, since nothing guarantees that a vaccine developed abroad, against another variety of the bacterium, will also be useful for us", emphasizes Ana Lucia. If the clues that the Brazilian researchers have picked up are shown to be correct and everything runs to plan, a form of immunization against the illness could become a reality in five to ten years time. To speed up the process, partnerships with the pharmaceutical industry and researchers in the veterinary area, which are also interested in new therapies against leptospirosis, are one of the priorities of the group from the Butantan Institute. •