

Death swarm

Brazilian group obtains the first serum against bee poison

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The expression “to fiddle with a wasp hive” indicates serious trouble. The same goes for bees. Bees are social insects that work together even when it comes to defending themselves from enemies. For this reason, people who hit one of these hives are most likely to land up in hospital with several stings on their bodies. And with toxins in their bloodstream for a couple of days, which damage the liver, kidneys and the heart especially, dissolving the tissue that binds the cells together resulting in chronic problems. “We have only recently been able to understand how the poison of these insects works,” says biochemist Mário Palma, from Ceis, the Social Insects Study Center of the State University of São Paulo (Unesp), Rio Claro campus. Palma sought reinforcements from the University of São Paulo (USP) and the Butantan Institute, and was able to put together a team that managed to develop a serum against bee stings.

According to Palma, the difficulty in producing a specific serum against insect stings was primarily due to a lack of knowledge of the composition of such substances. “Unlike serpents, whose poison is based on complex proteins, 70% of the bee and wasp poison is comprised of peptides,” he explains, referring to molecules related to the proteins, but smaller. He first noticed that these poisons work differently. A victim of a serpent bite – especially those that



The neotropical social wasp
(*Agelais pallipes*)



The *Polybia ignobilis* wasp

become meals, such as rodents – die quickly. After all, it is a hunting strategy. Bees and wasps, on the other hand, use poison as a defense mechanism: their fragile stings, though they only manage to penetrate the soft skin on the face of a monkey which is after honey, of a bird interested in insects or of an inattentive person, leave a very painful memory behind that marks the place to be avoided.

This year, the Ministry of Health believes that there will be from 10 to 15 thousand bee and wasp-related incidents – a vastly underestimated number, for people who are only stung once and do not develop a strong allergic reaction seldom seek medical assistance. Unlike serpents (over 20 thousand bites per year in Brazil), most patients survive. However, the small molecules of the insects' poison easily spread through the body. Thus, 98% of the victims of multiple stings suffer from sequela such as chronic kidney and liver problems.

Until recently, the method for finding serums and vaccines was based on trial and error: the serum was produced and its effect was tested. "A patient is lost every time it doesn't work," says the Unesp researcher. This result must be avoided even when laboratory rats are tested, but so far no one has been able to develop in vitro tests in order to assess the efficacy of the serums.

Palma's strategy was to build a laboratory with top technology to analyze proteins, with the help of a bioprospection project financed by FAPESP. The result is groundbreaking: in four years, Palma's doctoral student, Keity Souza Santos, co-supervised by Fábio Castro, found approximately 200 compounds in the bee's poison, in addition to the known proteins. But knowing the poison's composition is not enough; therefore, the researchers investigated its effect on the organism. On this issue, collaboration with University of São Paulo's

PHOTOS MARTELLI FILHO



The honeybee
(*Apis mellifera*)

(USP) Clínicas Hospital (HC), headed by immunologist Jorge Kalil and by allergist Fábio Castro, was essential. While caring for patients stung by bees or wasps, the physicians compiled a list of approximately 50 symptoms, which included pain, redness, edema, itching, darkening of vision, unconsciousness, fatigue in the legs and memory loss. The team was able to evaluate how each compound acts in the human organism by crossing this data with the list of peptides and proteins found in the poison.

Production - Palma then joined efforts with the Butantan Institute, responsible for the production of 80% of all the serums and vaccines consumed in Brazil. The Institute injected bee poison into its horses and extracted the response. Palma carried out *in vitro* tests at the laboratory in Rio Claro in order to verify whether the serum extracted from the horses neutralized all the toxic elements of the poison, and gradually added the missing defenses. “As far as we know, this process of looking for the antibody against each protein had never been carried out before,” he says.

In order to reach its final formulation, the serum also had to pass the approval of pharmacologist Marco Antonio Stephano, of the Pharmaceutical Sciences College of USP, specialized in quality control. “We had to keep all this a secret during the four years of work,”

says Palma, “until the request for the patent was filed”. Now that the formulation is ready, the Butantan Institute team headed by Hisako Higashi is producing the serum lots to be tested at the Vital Brazil Hospital, which is part of the Butantan Institute and a national reference center for accidents involving poisonous animals. The researcher estimates that the serum will be ready for clinical tests in approximately six months.

According to Hisako, in addition to enabling the proteins analyses, the partnership with Unesp is the source of significant quantities of bee poison to be used in the serum production.

The university maintains a bee farm for which biologist Osmar Malaspina, also a member of Ceis, takes responsibility. He places a glass plate covered by an electrified fence at the entrance of the hives. When the bees land, they receive a discharge, to which they react by stinging the glass. They do not actually lose their stings, but they leave a droplet of poison behind. With these droplets and the automatic method, Malaspina can get enough poison to produce the serum. Once approved, the product will probably be distributed to the entire public healthcare network. Palma says that this is a governmental



The *Synoeca cyanea* wasp



The wasp
(*Polistes lanio*)

undertaking, for it was financed by national research aid agencies – FAPESP, CNPq and Finep – and produced by Butantan Institute, which is under the State of São Paulo Health Bureau.

The Unesp researcher believes his first successful step is giving them the necessary energy to continue. The serum he developed works against Brazilian bees, but he has already received samples of bee poison from other places in the world to test whether it works against other subspecies of *Apis mellifera*, which can be found in 75% of the planet. If it does work, Palma already sees Brazil as the largest worldwide producer and exporter of serum against bee stings.

The team has not forgotten that bees are blamed for many wasp stings, whose poison is different and is not neutralized by the bee serum. With Malaspina's aid, Palma selected the 12 wasp species responsible for most of the accidents. The Rio Claro team is currently dividing the wasp poison into its peptide and protein components and is trying to produce a serum that works against all wasp stings, which are as dangerous as bee stings.

Allergy - Aside from being painful and toxic, the sting of one bee can bring on an allergic reaction capable of killing from one minute to the next. This happens because the immune system responds to the poison producing antibodies

called immunoglobins E, and or IgEs. When IgEs fight long battles against the poison, they cause edema, itching and even anaphylaxis in some people, which keeps them from breathing and causes immediate fainting. The serum has no effect against this reaction.

In order to fight an allergic process, its precise cause must be identified. Since in most cases one cannot demand strict scientific observations from the person suffering the attack, the Health Centers need tests to identify the allergen in the patient's blood. There are tests to detect allergens from certain North American and European wasps, but they are not the same species as found in Brazil. Moreover, the 51 spe-

cies alone found on the Rio Claro campus are greater than the European and North American biodiversity together. There are approximately 500 species in Brazil but only some 20 in the United States, and another 20 in Europe.

The team lead by Palma intends to develop tests to recognize at least the species that causes the largest number of accidents in Brazil, and to develop training programs to recognize and treat allergies to insect poison. "Most people who are currently trained to do so trained with us," says immunologist and allergist Fábio Castro, who is willing to train more professionals throughout Brazil. Castro and Palma have already started to expand their frontiers: they set up Genar, the New Regional Allergens Study Group, which intends to systematize a network of researchers and professionals in the health area to investigate and treat rare allergies, such as allergies to regional foods, about which little is known.

The project's success is an example of how scientific technology – in this case, the technology that enables the assessment of proteins and peptides – provides for surprising results when associated with knowledge of nature. "The animal toxins are true sources of inspiration," says Palma, who relies on spider and insect behavior and on the function of chemical substances in nature to understand how they act and how can they be used. ■

▶ THE PROJECT

Bioprospection of the arthropod fauna in the State of São Paulo for the development of new drugs and selective pesticides

TYPE

Biota Program

COORDINATOR

MARIO SERGIO PALMA - Unesp

INVESTMENT

R\$ 1,646,290.60 (FAPESP)
R\$ 1,530,000 (CNPq and Finep)