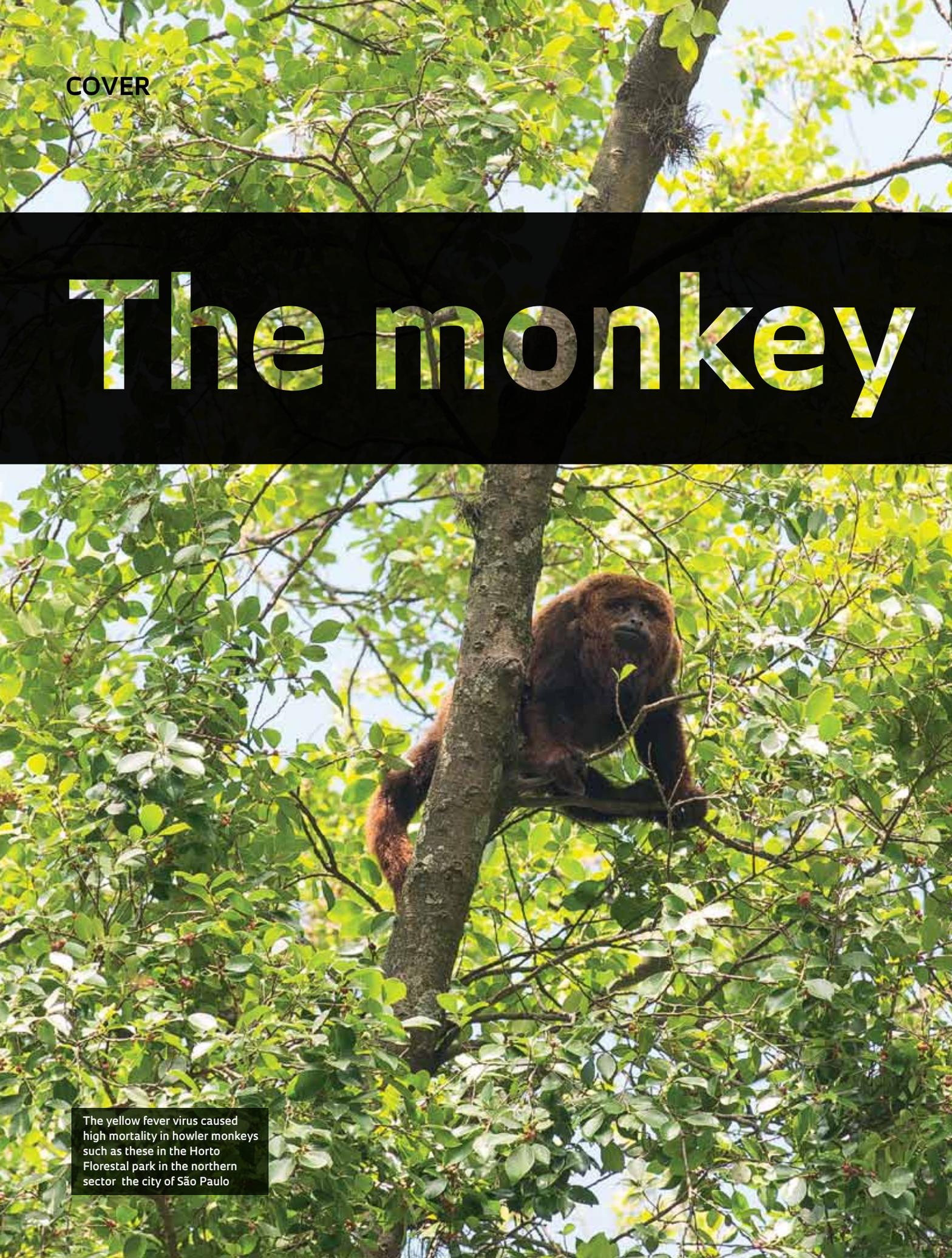


COVER

The monkey

A photograph of a brown howler monkey sitting on a tree branch, surrounded by dense green foliage. The monkey is looking towards the camera. The background is filled with sunlight filtering through the leaves.

The yellow fever virus caused high mortality in howler monkeys such as these in the Horto Florestal park in the northern sector the city of São Paulo

alarm

Even in cities where yellow fever is controlled by vaccination, the infection is decimating populations of wild primates whose deaths indicate areas where the virus is transmitted

Carlos Fioravanti | PUBLISHED IN JANUARY 2018

In the last three weeks of December 2017, ecologist Márcio de Carvalho, a researcher at the Forest Institute of São Paulo, collected 65 southern brown howler monkeys (*Alouatta guariba clamitans*) that were killed by the yellow fever virus in Horto Florestal, a state park in the northern area of the state capital, along with other biologists and teams from the Metropolitan Civil Guard and the Environmental Police. “Almost all the howler monkeys in the Horto died. We were familiar with all 17 groups,” he says.

In humans, the yellow fever virus can be fatal but can be halted by vaccination. However, in monkeys, which do not have vaccines, it is catastrophic. Public health agencies registered the deaths of more than 2,000 of these animals (mainly howler monkeys) during an outbreak that stretched from 2008 to 2009 in the state of Rio Grande do Sul; nevertheless, the virus’s effects are thought to be more extensive. Biologists and epidemiologists estimate that the number of wild primates reported to have died in urban areas from yellow fever corresponds to only 10% of the total wiped out by the disease. The other 90% die in wild areas,

decompose, and are never found. It is estimated that approximately 1,300 monkeys died in Espírito Santo and 5,000 in the state of São Paulo in 2017.

The deaths of the monkeys indicate the areas of greatest risk for transmission of yellow fever virus and guide vaccination campaigns (*see table*). “Without the monkeys, we are unprepared to perceive the arrival and movements of the virus,” warns biologist Júlio César Bicca-Marques, professor at the Pontifical Catholic University of Rio Grande do Sul (PUC-RS). “Before the monkey deaths were monitored, yellow fever mapping was exclusively based on people who became sick and died,” says biologist Renato Pereira de Souza, technical director of the center for virally transmitted diseases at the Adolfo Lutz Institute in São Paulo. “Only severe cases appeared, because people with milder symptoms did not go to the hospitals for treatment.” In 1999, the Brazilian Ministry of Health proposed that health agencies monitor monkey deaths as a strategy to identify new areas of virus transmission and to plan measures to protect people who live in cities, particularly near wild areas.

Sylvatic yellow fever is caused by a virus transmitted by *Haemagogus* and *Sabethes*, mosquitoes that feed on the blood of infected monkeys. These insects pass the virus on to new monkeys and, eventually, to humans who enter the forest. The monkeys do not directly transmit the virus to people. “Diseases such as yellow fever can cause the extinction of local primate species and should draw our attention because this type of threat combines with others, such as habitat loss and hunting,” says biologist Laurence Culot, a professor at São Paulo State University (UNESP) in Rio Claro. “The primates are victims two times over: victims of the disease, to which some species are very sensitive, and also victims of persecution by people who wrongly think that the primates cause the disease and kill them, thinking that this will solve the problem.”

Primates in the genus *Alouatta* (howler monkeys) are more sensitive to the virus and succumb more easily than those in the genus *Sapajus* (capuchins); both groups live in the Amazon and Atlantic Forests. *Callithrix* species (marmosets and tamarins), which are exclusive to the Atlantic Forest, have also proved resistant. As the virus circulates in forested areas, the animals continue to die of the disease, although approximately 20% of the total population survives by creating antibodies against the virus. The area for which vaccination is recommended for residents and visitors, previously limited to the Amazon, has grown and now covers nearly the entire country.

The yellow fever outbreak, which began in December 2007 and ended in April 2008, caused 40 confirmed human cases and 21 deaths. In São Paulo, 28 people were diagnosed, and 11 died of the disease. In the current outbreak, which is considered the largest in the last 14 years, between December 2016 and August 2017, 779 people were diagnosed and 262 died of yellow fever throughout Brazil, according to a December 2017 World Health Organization bulletin. Another bulletin, dated December 26, 2017, from the São Paulo State Department of Health (SES-SP), reported that 53 people were infected by the virus in the state, and 16 people died since the beginning of the year. Four more deaths were registered in the Greater São Paulo area as of January 9, 2018.

WAITING FOR THE VIRUS

The virus that caused the current outbreak is thought to have left the Amazon in 2014 and crossed the Midwest region to enter Minas Gerais and São Paulo through forest corridors. From there, it moved toward Espírito Santo, according to a recent study from the Endemic Diseases Oversight Office (SUCEN) and the Adolfo Lutz Institute. In São Paulo, the current outbreak emerged in April 2016 in the region of São José do Rio Preto



Veterinarians from the São Paulo city government begin necropsy on a howler monkey to collect organ samples and identify the cause of death

and moved to Campinas, leading to expectations that it would soon arrive in the capital (*see map*).

Biologist Juliana Summa, director of the São Paulo municipal Wildlife Division, noticed that, starting on the first Sunday of December, five to six dead monkeys began to arrive each day at the Wildlife Management and Conservation Center (CEMACAS) in Anhanguera Park in the northern zone of the city; this was three times the usual number, and it coincided with the intensifying summer rains and consequent proliferation of mosquitoes.

“Now yellow fever is strongly entering the northern zone of the city. Before there were just warnings that it would arrive,” Summa commented in the early afternoon of December 11. That day the center had already received five howler monkeys and a marmoset, all dead; by the end of the following week, another 12 had arrived. “We knew that the virus would reach the capital, but we can’t foresee everything,” says Summa. “In the beginning we didn’t know what to do with the young living monkeys that arrived with their dead mothers; they developed the disease within a few days and died.” The few animals that are alive upon arrival are quarantined; if they survive a week, they are transferred to the CEMACAS shelters.

Researchers from the University of São Paulo (USP), the city government, and SUCEN identified *Haemagogus leucoclaenus* and *Sabethes melanonymphe*, the mosquitoes that are the main transmitters of the yellow fever virus, in Anhanguera Park and the Cantareira Forest. “Mosquitoes feed on the blood of monkeys that live in the tree canopy, they only descend to the ground when food is lacking or the wind pushes them, and they randomly bite other animals includ-



Researcher at the Adolfo Lutz Institute prepares a liver sample from the necropsied animal to detect the yellow fever virus; below, samples stored at -70 °C



ing humans,” explains biologist Mauro Marrelli, professor at the USP School of Public Health.

The deaths of marmosets and capuchin monkeys in the interior of the state and howler monkeys in the cities near the capital have intensified collaborations among experts from research institutions and teams from the state and municipal departments of health and the environment, the Forest Police, and the Metropolitan Civil Guard. In June 2017, the Coordination of Sanitary Surveillance issued a statement defining the powers and procedures of the city’s Health Department teams. At the end of July, physician Helena Keiko Sato, director of immunization at SES-SP, gave a lecture to employees of public agencies and companies that work in the Horto Park, which is adjacent to another wildland area, the Cantareira Forest; the Cantareira is the largest urban forest in the country, spanning 80 square kilometers (km²) and the municipalities of São Paulo, Mairiporã, Caieiras, and Guarulhos (see Pesquisa FAPESP, *issue No. 207*). Sato spoke of the outbreak in São Paulo and the vaccination campaign, which was held at the end of August. The Forestry Institute’s Carvalho then spoke about the procedures to be taken when dead monkeys are found within or near the parks.

RAPID RESPONSE

Because he had attended the lecture, Manoel Ferreira Costa knew what to do on the morning of October 9 when he found a dead howler monkey in a eucalyptus plantation half an hour’s walk from the entrance to the Vila Amália arboretum, a section of forest belonging to the Horto that borders a neighborhood of approximately 3,000 inhabitants, where many areas are not walled off

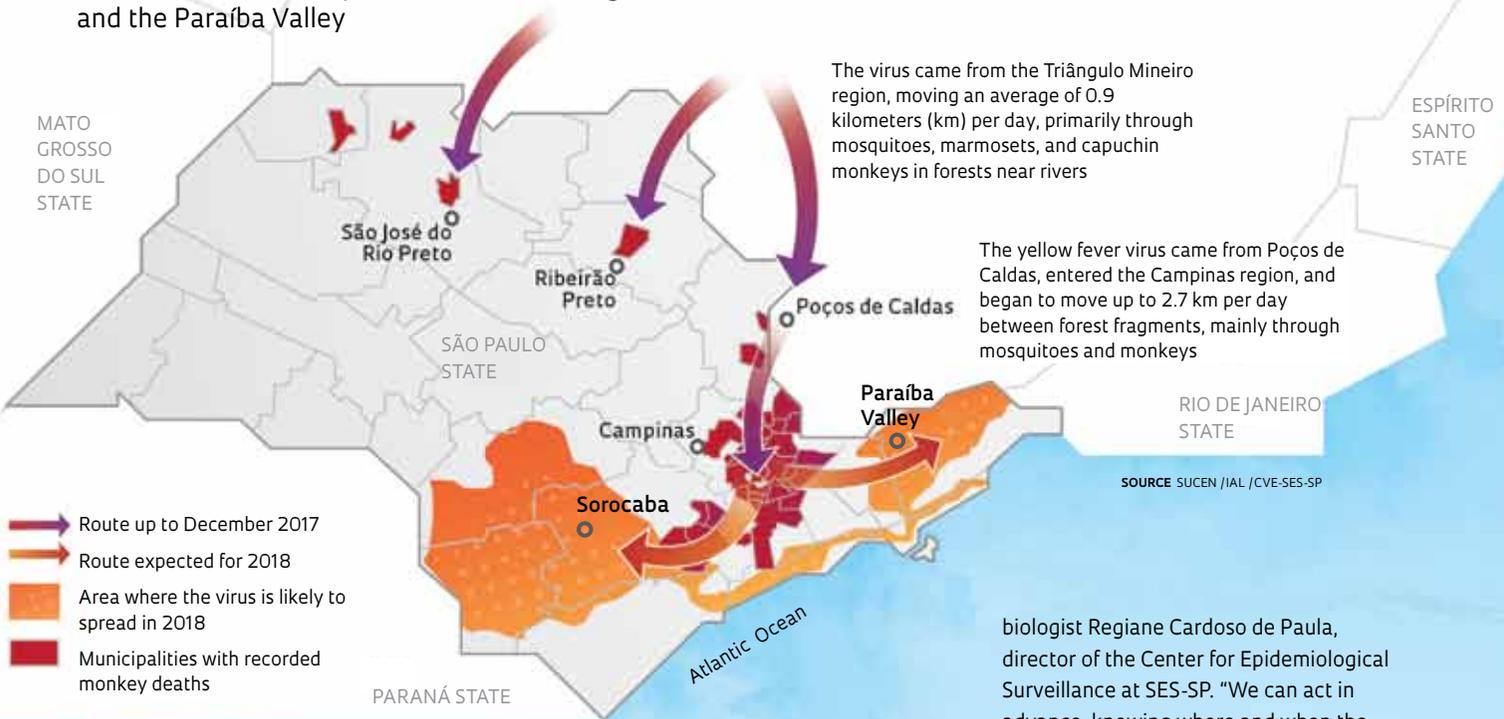
The 22 howler monkeys kept at Anhanguera Park could be strategic in repopulating the forests

and backyards merge with the forest. Carvalho and a park biologist, Paulo Roberto dos Santos, were advised and went to find the animal. Costa, who works in the park picking up trash and debris, accompanied them and saw that the monkey was a male less than a year old. There were no signs it had been attacked by dogs or other monkeys, electrocuted by power lines, or hit by a car, and it appeared to have died at least two days before. Carvalho officially advised Summa from CEMACAS, which received the animal soon after; she took samples from its liver, which she sent to the Adolfo Lutz Institute that same day for analysis.

At Adolfo Lutz, Souza has been receiving organ samples from dead howler monkeys in the state of São Paulo since 2016, but he paid special attention to this request for examination since it was the first from a city that had not yet shown signs of the yellow fever virus. His team extracted the DNA, conducted the exams, and then reran them to confirm the positive results for the virus. On the morning of October 19, he reported the results to medical biologist Regiane Cardoso de Paula, director of the Center for Epidemiological Surveillance at SES-SP. De Paula, in turn, immediately took the results to infectologist Marcos Boulos, coordina-

Yellow fever routes in São Paulo

In 2018, the virus is expected to reach the region of Sorocaba, the coast, and the Paraíba Valley



Getting there before the virus

Strategy hastens vaccination of residents in areas of risk

On the basis of the dates and locations of the monkey deaths, veterinarian and epidemiologist Adriano Pinter, a researcher at SUCEN, constructed an epidemiological model that describes the direction, velocity, and probable paths (functional ecological corridors) of the virus that causes yellow fever. His maps support the state Department of Health's decision to set aside the strategy recommended by international bodies, which is to vaccinate all residents within 30 kilometers (km) of where a dead animal was found and to vaccinate only the inhabitants of risky areas, even before the dead monkeys appear and indicate the arrival of the virus.

"This strategy has been shown to be sufficiently adequate," says physician Helena Keiko Sato, technical director of the immunization sector of SES-SP. "In April 2017, we were unable to vaccinate 3.5 million people in the Campinas region, most of them outside the areas of risk. Based on

the ecological corridors, we vaccinated only 1.4 million, in the high-risk areas of Campinas and neighboring municipalities." Not only does this strategy optimize the use of vaccine stocks, it could also reduce potential severe reactions to the vaccine in people who have autoimmune diseases or egg white allergies; the risk of severe adverse reactions is 1:400,000, four times less than the acceptable rate for vaccines.

By January 2018, the virus had moved 2.7 km per day in a north-south direction during warmer months and 0.5 km per day in colder periods. On the basis of this information, specialists from the Department of Health define the areas of greatest risk and begin vaccination, in collaboration with municipal health departments, before dead monkeys appear. "In Jundiaí, vaccination began at the beginning of May and the first dead monkey was found on July 30. In Mogi das Cruzes, we still have no sign of the virus, but vaccination began in December," says the medical

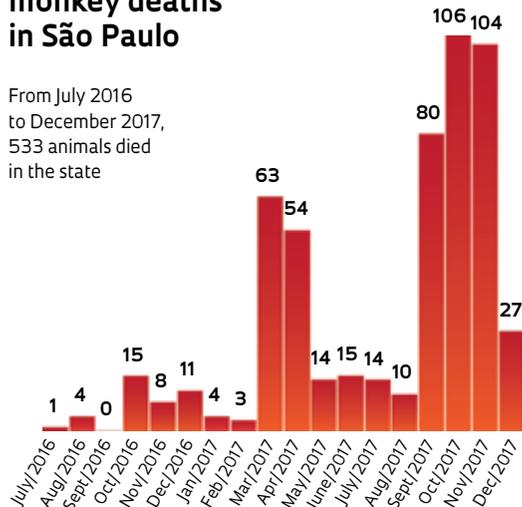
biologist Regiane Cardoso de Paula, director of the Center for Epidemiological Surveillance at SES-SP. "We can act in advance, knowing where and when the virus will arrive."

The epidemiological model forecast the arrival of the virus in the capital of São Paulo in October or November. "We were lucky to find a dead howler monkey in the middle of a forest in the city of São Paulo," commented Pinter. He says that the first animals infected by the virus die in the woods and go unnoticed. The virus is only noticed about two months after it arrives, when many animals begin to die at the edges of the forests and are seen by residents in the suburbs. The fact that an animal was found in October in the Horto Florestal park moved virus preventive measures forward.

Health agency teams expect to prevent further human deaths through vaccination campaigns in the areas where the virus is likely to spread in 2018 (see map). If the forecasts are correct, the virus will arrive in the area south of the capital in February in the region of Sorocaba and the Paraíba Valley. SES-SP announced in January that it would divide the vaccine doses to vaccinate as many people as possible without reducing its effect, which has already been done in Africa. Anyone who lives or circulates in wooded regions should get the vaccine, which activates the production of antibodies against the virus only seven to ten days after it is applied.

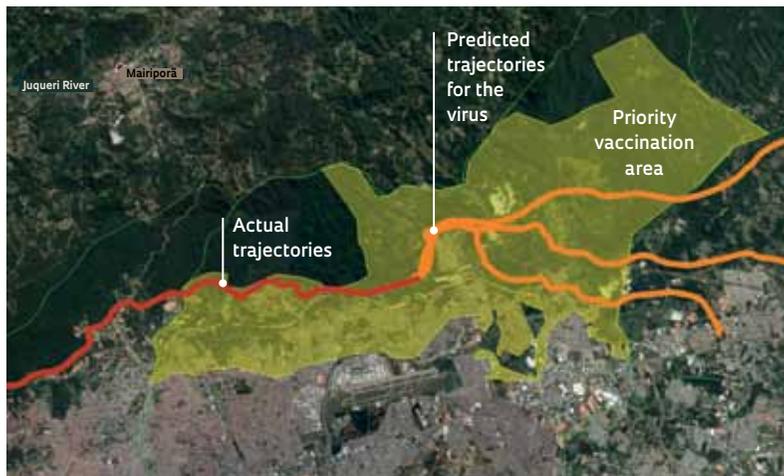
Reported monkey deaths in São Paulo

From July 2016 to December 2017, 533 animals died in the state



Priorities for vaccination in Guarulhos

Actual and predicted virus trajectories define areas that are priorities for vaccination



tor of the SES-SP Centers for Disease Control and professor at the USP School of Medicine.

A day of meetings between health and environmental teams ended with two decisions: immediately begin vaccinating residents in the areas near the forest where the monkey was found and close the Horto and Cantareira areas to avoid human contact with the mosquitoes that transmit the virus. On October 20, shortly after the parks were closed, teams from the Forestry Institute, SES-SP, and the Environmental Police returned to the arboretum and found three more howler monkey carcasses; two days later, two more were found, indicating that the entire band was dead. In the last week of December, after 10 monkeys were found dead in the municipality of Itapeperica da Serra in the southern São Paulo metropolitan area, 10 other parks were closed, totaling 26.

REPOPULATION

The monkey deaths are expected to continue until the rains wind down in May, hampering the proliferation of mosquitoes that transmit the virus. “The next battle will be to repopulate the areas which were previously occupied by howler monkeys,” says Juliana Summa. The 22 animals that were maintained in 18-square-meter enclosures at CeMaCAS may be strategic in repopulating the forests. Báculo is the oldest and most established monkey in his band. He arrived in 2009 as an adult, and he formed a family composed of a female, a young male, and an infant, which all could be released in the depopulated areas. Others, such as 6-year-old Abrolhos and 5-year-old Benjamin, arrived as juveniles and would have to be trained to survive in the forest.

The outbreak of yellow fever in 2008 and 2009 caused losses of 80% in groups of black and

southern brown howler monkeys in Rio Grande do Sul, according to a study by PUC-RS and the Federal University of Santa Maria covering 82 fragments of forest in the towns of Bossoroca and Santa Maria. “We did not find solitary individuals, indicating that the entire group had died,” reported Marques. In 2009, Marques launched a campaign to protect howler monkeys from attacks by those who believe that the monkeys transmit yellow fever, which was described in 2010 in *Tropical Conservation Science*.

If no other similar epidemics occur, the population of howler monkeys in Rio Grande do Sul may take 100 years to reach half of what it was prior to 2008, according to the team from Santa Maria. Primate loss is a worldwide problem; according to a 2017 study in *Science Advances*, 75% of the 504 species of primates in the world (which are concentrated in Brazil, Congo, Mali, and Indonesia) are facing population decline, and 60% are at risk for extinction as a result of deforestation, hunting, and disease. ■

Project

Biodiversity of mosquitoes (Diptera: Culicidae) in Cantareira State Park and the Capivari-Monos environmental protection area in the state of São Paulo (No. 14/50444-5); **Grant Mechanism** Regular Research Grant; **Principal Investigator** Mauro Tadeu Marrelli (USP); **Investment** R\$272,905.54.

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