

# Chagas disease in the Chaco

Researching disease transmission in poor, rural settings is part scientific inquiry, part diplomacy.

**Y**ou would not know it if you drove to his small farm, but Ramón Raúl Alegre is the owner of a unique mud hut. It looks like all the other adobe outhouses dotted near the dusty, dirt tracks in Pampa del Indio, which is a municipality of Argentina's northerly Chaco province, except that it is more dilapidated than most. That is because the walls of Alegre's hut have been dismantled chunk by chunk, every few months, by scientists from the University of Buenos Aires (UBA) in Argentina, who are searching for the blood-sucking bug *Triatoma infestans*. It is the region's main vector of *Trypanosoma cruzi*, which is the parasite that causes Chagas disease.

The reason for this piecemeal destruction is that Alegre's hut is the first known to house bugs that somehow developed resistance to pyrethroids, the class of insecticides that has dominated vector-control programmes for Chagas disease since the mid-1980s. Curiously, the bugs developed this resistance in the absence of repeated exposure to pyrethroids. No one is sure how this happened. It is possible that the bugs were recently transported from a site in the western part of the Gran Chaco — a vast lowland plain that encompasses parts of Argentina, Bolivia and Paraguay — or that the resistance arose spontaneously in the local population.

The initial sign came when Alegre was bitten at sunset in a nearby field shortly after

the hut was first sprayed. This prompted an experiment. Four times at 2-monthly intervals Juan Manuel Gurevitz, a PhD student at the UBA, re-sprayed the hut with pyrethroids and collected the bugs that died. Between each application, Gurevitz placed a tent of mosquito netting over the whole structure to stop bugs escaping or re-infesting the hut — and to prevent Alegre's goats from gobbling fallen insects.

"We tried all approaches to maximize pyrethroid effectiveness," says Uriel Kitron, who is a professor of environmental sciences at Emory University in Atlanta, Georgia, in the United States, and who is observing the study site. "All the time the bugs were able to reproduce and recover their numbers." Today's inspection suggests that the fifth spraying in 2 years, this time with malathion — a much more toxic insecticide that cannot be purchased over the counter in Argentina — has finally nailed Alegre's problem. The pyrethroid resistance has been confirmed by blind laboratory tests on the bugs.

## Life-long Chagas man

Both Gurevitz and Kitron work with Ricardo Gürtler. Now aged 55 years, Gürtler has studied the fundamentals of Chagas disease transmission and control since he was a PhD student. For roughly the first half of his career, Gürtler watched with optimism as the big budget, militaristic Southern Cone Initiative interrupted vectorial transmission of Chagas disease in three of Argentina's five neighbouring countries. This happened in a few provinces of Argentina as well. After 2001, when the country experienced the largest sovereign default in economic history, however, Argentina's control programmes were degraded by inconsistent funding and vague or absent management. The number of Argentines displaying acute symptoms of Chagas disease has since risen in eight of the 22 provinces.

The bulk of Gürtler's research comes from Santiago del Estero province in Argentina, where he and his co-workers kept tabs on a study site between 1984 and 2006 (ref. 1). They have compiled a huge database. Over the years,



they monitored bug densities, counted how many bugs were infected at different stages of development, tested dogs, cats and residents, and organized communities to plaster the walls of some houses to eliminate the crevices that the bugs like to hide in. They used the information to model *T. cruzi* transmission mathematically<sup>2</sup>. Policy recommendations fell out of their models.

"For example, Ricardo showed that if you have fewer than two dogs in a household, you're unlikely to get infected," says Rick Tarleton, who is a professor at the University of Georgia and has researched Chagas disease since the 1980s. "That information has been helpful in planning dog vaccination programmes." A group at the Autonomous University of Yucatán, in Mérida, Mexico, has started testing a therapeutic DNA vaccine against *T. cruzi* in mongrel dogs. In 2008, they published the results of a pilot clinical trial, which showed some success. Over the past decade, researchers have increasingly used dogs as sentinels of *T. cruzi* transmission, and new research has suggested that their immune reaction against bug salivary antigens can be used as a surveillance tool.

"The dog issue is a classic example of the kind of heterogeneity we keep finding with Chagas," says Kitron, as a ragged old dog limps past. "This guy's much more likely to be the



Living *T. infestans* collected from a chicken coop near Alegre's unique mud hut.





**Alegre's unique mud hut, partly dismantled for science.**

source of infection than the other dogs here.” Similarly, 20–30% of the buildings in each community seem to act as foci of infected bugs that rapidly re-infest other buildings after a spraying campaign<sup>3</sup>. “But that means that control can be targeted,” adds Gürtler.

The Santiago del Estero project revealed the basic dynamics of Chagas disease in the Gran Chaco. Now that job is done, Gürtler's focus has been to understand how they differ across it. For this, he has established three new study sites in the Gran Chaco: one in Bolivia, one

in Paraguay and one in Argentina. The sites have different vegetation, climate and ethnic groups. An added bonus of the new set-up, Gürtler hopes, will be to encourage more collaboration between academics and vector-control officials in the three countries.

### **Tobas and triatomines**

Pampa del Indio is Gürtler's new location in Argentina. It has a population of ~15,000. One-third of the inhabitants live in a town of the same name; the rest live in huts scattered

along the few tracks that transect the municipality and along the edges of scrubby forest. It is among the poorest municipalities in Argentina, and has probably the highest percentage of people of indigenous origin of any municipality in the country. The local indigenous folk in Pampa del Indio belong to the Toba ethnic group. Tobas lived a largely nomadic life, hunting and fishing, until deforestation and agriculture arrived in the area in the early decades of the twentieth century. The government of Juan Perón, who was Argentina's famous thrice ruler, gave some Tobas land titles ~60 years ago. This encouraged them to live a more agricultural existence.

Alegre is Toba. His other hut — where he lives — shows why Toba homes are much more likely to be infested with *T. infestans* than those of Creoles in Pampa del Indio. Tobas often choose to sleep on the floor. They tend to thatch the roofs of their houses, providing *T. infestans* with more prime habitat than just mud walls. In general, Tobas are poorer than the non-indigenous inhabitants, so they can less easily afford insecticides, and find it harder to travel to the town's small hospital where they might learn about Chagas disease and receive treatment. The Toba population is also in constant flux: Tobas are attracted to Pampa del Indio because it is where their community is gaining political power, which is also why the town acts as a springboard for those migrating to Argentina's main cities seeking work or bigger welfare payments.

For these reasons, monitoring *T. cruzi* transmission among indigenous people is often difficult. “Sometimes the Toba ask ‘Why do you want my blood?’” explains Gürtler, who has taken to first testing households' dogs and presenting these results to the owners to convince them that a blood test is in their interests. “You can't take samples from people and leave. You have to build trust — it takes

## Box 1 | Understanding the human element

It is politically unfortunate that most of the known foci of pyrethroid-resistant *Triatoma infestans* are in the western Gran Chaco, in Bolivia. Bolivian immigrants are frequently and mistakenly blamed for many of Argentina's self-wrought woes. The discrimination is such that one Argentine news outlet recently reported that “two people and a Bolivian” had died in a car crash. Ricardo Gürtler has come across this in his own research.

In 2007, he and entomologist Delmi Canale of Argentina's national Chagas disease programme travelled to the Patagonian province of Chubut to see whether *T. infestans* still existed so far south. They went on the invitation of the local officials, who were concerned about the number of Bolivian migrants in their province testing positive for *T. cruzi*. Gürtler and Canale could not find any *T. infestans*. “But a few months after I went there, an adult bug appeared in a health-care

centre right in the middle of the city of Comodoro Rivadavia. It was found by a cleaner who said that Bolivians had been to the centre a few days earlier,” recalls Gürtler.

Slowly the insect was sent from official to official in Chubut. Eventually it was mailed to Gürtler, dead but having laid several eggs in its plastic container. Gürtler and Romina Piccinali, who was one of his post-doctoral researchers, used their database of the common haplotypes of *T. infestans* to compare the sequence of the

Patagonian bug's mitochondrial cytochrome oxidase I gene to typical DNA sequences from all over the Gran Chaco. “It was a 100% Argentine bug, probably from the western provinces of San Juan, Mendoza or Rio Negro,” Gürtler says through a wry smile. Shortly afterwards he returned to Comodoro Rivadavia and discovered that most of the Chagas-carrying migrants in the town were actually from those three Argentine provinces, and living in a middle-class neighbourhood.





Gürtler (left) and Alegre (right).

time — and the local health-care staff can't do this because they are understaffed."

Tobas also have a different attitude towards their homes. For instance, last year, Gürtler and his team found 500-odd vectors in one house, just 15 min after spraying — which suggested that the hut could have been infested with ~5,000 bugs in total. Rather than treating the house, the family decided to burn it to the ground. "They often do the same if a relative dies in a house — burn it and build another house close by," explains Gürtler. Understanding these idiosyncrasies is part of what he calls "the human element", which Gürtler believes is neglected by most Chagas disease control programmes — a sentiment echoed by on-the-ground researchers in other countries (Box 1).

### From Chagas to Kafka

This trip's purpose is to show Kitron around the new study site. As part of the tour, Gürtler drops in on the local hospital. It seems clean, with a committed but under-trained staff. The director, Arturo Navajas, is open about its problems. He does not have good statistics on the prevalence of Chagas disease locally. His best data are for the pregnant women who visit the hospital, of which ~10% test positive. The true figure is almost certainly higher. Moreover, the hospital has only two boxes of drugs — enough for merely 15 people — that arrived a year after they were ordered.

Inefficiencies at every level beset the scientists trying to study Chagas disease. In 2002, in the middle of Argentina's economic crisis, Gürtler and Kitron received a grant from the United States National Institutes of Health, of which more than US\$500,000 went to their work in Argentina. Meanwhile, to reduce the rate at which cash left the country, the Argentine government passed a law limiting the amount

of money that could be withdrawn from bank accounts to a few hundred dollars a week. This meant that Gürtler had to buy reagents and minor equipment during trips to the United States. "Even today we have to justify every dollar that enters the lab's bank account as if we were laundering money. It's really not anyone's fault," he shrugs. "It's the way everything works here. It's a kind of Kafka-esque story. People from abroad find it hard to understand. It's a challenge everywhere to get funded [...] but here dealing with how you get to the money takes about a third of researchers' time."

Like many countries in Latin America, Argentina has a decentralized public-health system. Provinces and municipalities administer vector control in a whimsically individual way. In Pampa del Indio, for example, no official is responsible for ensuring that houses are sprayed with insecticides; however, favours can sometimes be bartered. The morning after Gürtler shows Kitron the hospital, he is back to speak to Navajas. He has telephoned Hector Frelj, who is a friend of his in Buenos Aires and who has the difficult task of coordinating Chagas disease control at the national level. Frelj has agreed to send more drugs directly to Pampa del Indio. In response, Navajas says he will help Gürtler in organizing cross-sectional blood tests of the community to provide an estimation of the local prevalence of the disease.

### The dark side of data

Good data are scarce, and the more scientists dig, the more they seem to be debunking the assumptions upon which the Southern Cone Initiative was based. In the beginning, eliminating *T. infestans* was considered achievable because this vector was thought to be highly susceptible to pyrethroids and lacking the

genetic variation required to develop resistance. Several groups, including Gürtler's, have disproved these assumptions. The bugs were also thought to be bad fliers and unable to re-infest from afar. Gürtler's data from Santiago del Estero, however, reveal that they can travel distances of 500–2,000 m<sup>4</sup>.

It was also assumed that once all of the *T. infestans* bugs in a settlement had been eliminated, re-infestation from nearby forests was impossible. Except for a melanic version or 'dark morph' of *T. infestans* that lives in the Andean valleys of Bolivia, *T. infestans* was thought to be unable to survive and breed in the wild. Again, this is untrue. In the summers of 2006 and 2007, several members of Gürtler's group identified dark morphs in forest 40 km from the town of Fuerte Esperanza in northeast Argentina, not far from Pampa del Indio<sup>5</sup>, and ~1,000 km from the Andean valleys of Bolivia. They did this by piggy-backing on a study of the local population of *Amazona aestiva* and *Aratinga acuticaudata* parrots. These birds tend to nest in hardwood trees with trunks made hollow by a fungus. When Gürtler's group placed jars containing live mice (as bait) in the trees, they recovered dark morphs of *T. infestans* glued to the sticky paper placed around the necks of the jars. The trapped bugs appear to be genetically much closer to the insects living in huts in Pampa del Indio than to the sylvatic dark morphs in Bolivia — suggesting that some of the re-infestation in the eastern Gran Chaco comes from non-domestic populations.

"It's just that no one looked for them before," suggests Gürtler. Similarly, nobody thought to study urban vectors until recently — or systematically checked, after spraying campaigns, whether the bugs really did die. These revelations point to why the original Southern Cone Initiative did not work everywhere, and give some hope that programmes can be designed to reinvigorate it.

Back at Alegre's unique mud hut, Gurevitz has found *T. infestans* vectors in a nearby chicken coop. It is unknown whether these originated from the resistant population in the hut or flew to the coop from another house 500 m away. Gürtler, Gurevitz and Kitron drive to Alegre's house to try and find him. His wife appears. "More bugs?" she asks, disappointed. "Don't worry, we'll get rid of them. We'll be back," promises Gurevitz. ■

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- Gürtler, R. E. *et al. Proc. Natl Acad. Sci. USA* **41**, 16194–16199 (2007).
- Cohen, J. E. & Gürtler, R. E. *Science* **293**, 694–698 (2001).
- Cecere, M. C. *et al. Emerg. Infect. Dis.* **12**, 1096–1102 (2006).
- Vazquez-Prokopec, G. M. *et al. Med. Vet. Entomol.* **20**, 1–6 (2006).
- Ceballos, L. A. *et al. J. Med. Entomol.* **46**, 1195–1202 (2009).