Glasgow, United Kingdom 18th July 2018

Where the kissing bugs are

A field report from the Ecuadorian highlands, funded by the BSP International Training and Fieldwork Award 2018

By Luis Enrique Hernandez Castro

I started my PhD studies in Molecular Epidemiology at the University of Glasgow in 2016 supervised by Dr Martin Llewellyn and Dr Louise Matthews. Since then, I have been either working at the molecular wet-lab bench or analysing NGS data at my desk exploring the population dynamics and genomics of triatomine insects, commonly known as kissing bugs. They transmit the protozoan parasite, *Trypanosoma cruzi*, after post-bite contamination with infected faeces, causing Chagas disease mainly in Latin America. Although I have gained many practical and analytical skills as a lab/office based student, I have always believed that visiting where the kissing bugs are will complement my understanding of Chagas disease as a system with multiple biological, ecological, social and epidemiological aspects.

The BSP international training and field work award allowed me to join the Chagas disease research group at the Pontifical Catholic University of Ecuador (PUCE), led by Dr Mario Grijalva (PI) and Dr Anita Villacís (entomologist), to their 2018 summer field trip in Loja, Ecuador (Figure 1) for triatomine bug collection and house insecticide spraying. What I found during this 2018 fieldwork trip was beyond my imagination, I can say with no doubt it has been a life-changing experience and it has given me a reality I never considered before for understanding both my Chagas disease system and kissing bugs dispersal, key concepts for my research.

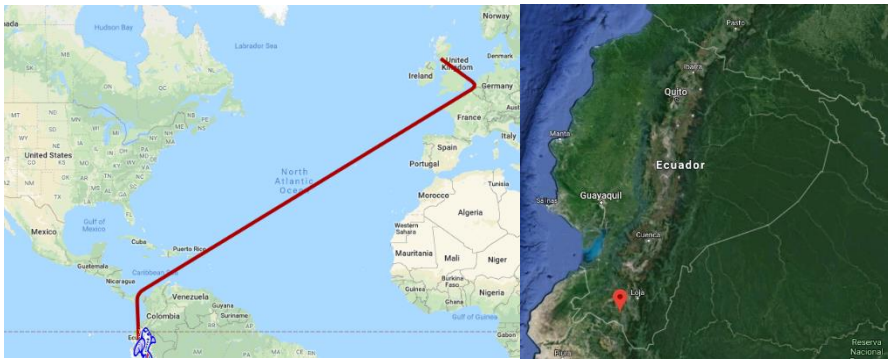

FIGURE 1 DISTANCE TRAVELLED AND LOCATION OF CARIAMANGA (RED MARK) WITHIN LOJA PROVINCE IN SOUTHERN ECUADOR.

I travelled approximately 5,860 miles from Glasgow, United Kingdom to Loja, Ecuador in the southern highlands. As a Latin American, born and breed in southeast Mexico, the weather, language, food and the Latin American way of life was not a big shock for me. I must say though, the nostalgic for being nearly two years away from home started to emerge very soon after I landed, that and the high-altitude dizziness. I was transported from Loja city to Cariamanga (Figure 1), town 63 miles in the south, where the team was based. Soon after my arrival in Cariamanga, I met with Soledad Santillan, a research assistant who explained me the dynamic of the everyday work. She strongly encouraged me to have the essentials for the field: a hat/cap, long sleeve t-shirt, comfy shoes, dust-mask, UV sun protection and lots of water. At first, I though a hat/cap might be too much, but next day in the long walks under a burning sun I was very thankful I did not listen to my stubborn self and I did spend the previous evening looking for a cheap yet stylish cap (Figure 2) all around Cariamanga.

FIGURE 2 SOME OF THE VIEWS IN THE COMMUNITIES IN LOJA AND MYSELF WITH MY CHEAP STYLISH CAP.

During the fieldwork, the daily routine started as early as 5:30 am with the craw of the rooster when all the team including local and international students and researchers, research and field assistants, Ecuadorian ministry of health staff, administrative and logistics staff, translators and van drivers gathered for “el grito de guerra” (the scream of war, Figure 3). This was an old tradition starting back in 2002, where according to PI Mario Grijalva’s own words it was a reminder of “why everybody was there and why we were doing what we were about to do in the field” and after that everybody replied loudly and with passion “with our work, Ecuador without Chagas”.

FIGURE 3 EVERYBODY GATHERED FOR "EL GRITO DE GUERRA" AND THEN ENJOYING BREAKFAST BEFORE HEADING TO THE FIELD.

After a full Ecuadorian style breakfast, we were ready to head to the rural communities which normally took one-hour drive up and down hills and mountains in very difficult roads (Figure 4). Once in the communities and given their isolation, an improvised camp was set for emergencies, having lunch and rest before heading back to Cariamanga. I was part of the entomology team; therefore, I was assigned to different groups every day formed with more experienced staff mostly from the ministry of health and PUCE. We aimed to cover approximately 80 houses on each community spending three to four days on each one, and visiting six communities in total.

FIGURE 4 DIFFERENT MOUNTAIN ROAD ROUTES DRIVEN IN ORDER TO GET TO THE COMMUNITIES.

At the house (Figure 5), the first step was to explain our procedure to the house owner, obtained his/her ethical approval and permission to access the house, and fill in socioeconomic and entomologic surveys. Then, we took authorised pictures of the house, collected GPS readings, proceeded with indoor and outdoor kissing bug searching and finalised with insecticide spraying. Inside the house (Figure 6), we searched under mattresses, in-between bedding, accumulated clothing and objects, walls cracks and under walls-hanging objects. Chicken and guinea pig nests, inside and outside the house, were inspected at first as they were more likely to be infested than other domestic animal beds. Mask and latex gloves were used always for searching and insecticide spraying. All collected kissing bugs were deposited in labelled plastic containers and separated according to the collection site. In domestic settings, both *Rhodnius ecuadoriensis* and *Panstrongylus chinai* were frequently found and deposited separately no matter their life stage.

FIGURE 5 MAIN STEPS IN DOMICILE PROCEDURE: SURVEYING, TRIATOMINE SEARCHING AND INSECTICIDE SPRAYING.

FIGURE 6 INSIDE OR OUTSIDE LOCATIONS IN THE HOUSE WHERE KISSING BUGS CAN BE FOUND SUCH AS GUINEA PIG AND CHICKEN NESTS, UNDER THE BED, WALLS CRACKS AND ACCUMULATED MATERIALS.

In the wild (Figure 7), we looked for squirrel, bird and rat nests hanging on tress, or inside bushes and palm trees. When a nest was in a safe access point, the so-called monkey men, very skilled climbers of the team, proceeded to reach the nest and placed it on an unfolded plastic bag for inspection, latex gloves and masks were wore always. As in domestic procedures, GPS readings and pictures of the nest were taken and the collected bugs were placed inside labelled plastic containers, however, in the wild only *R. ecuadoriensis* bugs were found. After inspecting and collecting as many bugs as possible, the nest was carefully burned to kill left bugs. Once all teams were finished for the day and were back from domiciles and the wild, all bug containers, surveys, GPS and radios devices, and digital cameras were stored and the camp was raised.

FIGURE 7 NESTS WERE REMOVED FROM THE TREES FOR INSPECTION. *R. ECUADORIENSIS* EGGS, NYMPHS AND ADULTS WERE FOUND AT DIFFERENT LOCATIONS IN THE WILD.

Back in Cariamanga, a laboratory (Figure 8) was set to observe kissing bugs faeces under the microscope to confirm *T. cruzi* infection and an approximate infection rate was calculated. Moreover, faeces samples were taken for blood agar *T. cruzi* culture at the research centre in Quito, Ecuador. Antennae, wings and legs samples were also taken for morphological studies and the rest of the body was preserved in alcohol for future triatomine genetic studies. Alive specimens, such as nymphs, were also kept and brought back to the facilities in Quito to grow triatomine colonies.

FIGURE 8 FAECES, ANTENNAE, WINGS, LEGS AND BODY SAMPLES WERE TAKEN FROM THE KISSING BUGS COLLECTING IN THIS FIELDWORK AND PARASITE PRESENCE WAS CONFIRMED (RED ARROWS) UNDER THE MICROSCOPE.

Describing experiences, skills and knowledge gained during this trip could be endless and in more detail, however, I hope the previous paragraphs provided the reader with an impression of what fieldwork in one of the many Chagas disease systems involves. From now on and while analysing my data, I will have a complete picture of the situation of Chagas disease in Ecuador. Picturing the face of that sweet old lady where we found more than 20 bugs underneath her bed or of those little children constantly bitten by bugs will motivate me to make the most of my research findings and keeping in mind “with my work, Ecuador without Chagas”.

I would like to thank my supervisor, Martin, who has been an inspiration and very supportive throughout my PhD studies and all the team in Ecuador for teaching me how to do Chagas disease fieldwork. I am also very thankful with the people of the communities for cooking an Ecuadorian delicatessen, fritada de chancho, as a thank you for our work in their homes (Figure 9). Finally, thank you to the British Parasitology Society for their support for this fieldwork.

FIGURE 9 PARTY ORGANISED BY THE PEOPLE FROM THE COMMUNITIES AS A THANK YOU FOR OUR WORK.