II

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"Control of endemic zoonotic and vector-borne emerging and re-emerging diseases: Current challenges in Latin America"

ABSTRACT BOOK

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ABSTRACT BOOK

of the

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"Control of endemic zoonotic and vector-borne emerging and re-emerging diseases: Current challenges in Latin America"

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Chagas: a comprehensive look for an effective approach

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When we talk about Chagas in an academic field of biomedical sciences it is usually understood in terms of "Chagas disease", and we expect entomological, clinical, diagnostic aspects, or number/distribution of affected people, to be addressed, to name a few. On the other hand, if social, political, economic, productive, educational, communicational, ethical, inclusion, diversity and gender aspects were included, it would probably come as a surprise. Nevertheless, these topics are also part of the Chagas matrix and affect its comprehensive approach because Chagas is much more than a disease: it is a complex socio-environmental health problem. To illustrate this idea, the Group "What do we talk about when we talk about Chagas?" (*¿De qué hablamos cuando hablamos de Chagas?*) uses the metaphor of a "*kaleidoscopic puzzle*" where, as in every puzzle, each *piece* is essential for completing the image and, as in every kaleidoscope, each *piece* contributes its own shape, colour, and size, dynamically interacting with the rest of the pieces to intervene and complete the image. This kaleidoscopic analogy helps us understand our scientific contribution as a *piece* of a more comprehensive cultural and collective construction, in which knowledge originates from many sources. Furthermore, when we analyse our scientific activity in a fractal way, we can see that this is also made up of *pieces* of different nature, such as our object of study, where we work (group, institute, infrastructure, funds, etc.), our motivation, our driving-values, and our ethical and epistemic position. Even though in Argentina it is mandatory and a responsibility of the Health System to test and detect children born with Chagas, only 25% of the estimated cases are diagnosed annually. Because of this, our R&D Group (ICT Milstein) worked to improve the diagnosis of neonatal Chagas exercising a comprehensive view with gender perspective. We focused on developing and transferring a simplified and affordable molecular test, which could be carried out in any condition, reducing infrastructure, equipment, and HR requirements to a minimum. The result was the first Argentine molecular test approved by the National Health Authority (National Administration of Drugs, Food and Medical Technology, ANMAT). The test is based on a LAMP reaction, it does not require DNA extraction, and can be performed using the neonatal screening card (a mandatory neonatal sample taken to screen for congenital diseases). The benefits are manifold: not adding another intervention on the newborn, nor another sampling/extraction activity on the Health System, and reducing neonatal diagnosis times (which generally extend beyond 10 months of age) and follow-up failures (which are responsibility of the Health System, not of the mothers or families). This is only one example of how reassessing our scientific activity with an integrative perspective can help us compose a more complete image of Chagas (or whichever topic we work on) to achieve a comprehensive, situated and effective approach.

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Kdr mutations in mosquitoes *Culex quinquefasciatus* and *Aedes aegypti* from urban Houston and their influence on survival after Permanone® field bioassays

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Arboviral surveillance in Harris County (TX), which includes the City of Houston, is performed routinely by the Harris County Public Health Mosquito and Vector Control Division. Upon detection of West Nile virus (WNV) or other arboviruses in any of the 268 mosquito control operational areas in which Harris County is divided, pyrethroid applications of Permanone® 31-66 (hereafter Permanone®**;** Permethrin 31% + PBO 66%) are carried out, alternating with ULV malathion on a weekly basis, as needed. The objectives were to identify specific *kdr* mutations in the voltage-gated sodium (Na$_v$) channel of single female mosquitoes of *Culex quinquefasciatus* and *Aedes aegypti*, develop a PCR method to detect *kdr* in *Cx. quinquefasciatus,* and assess the impact of the *kdr* mutations on the probability of survival after a control field application of Permanone® for both species. Field cage bioassays were performed using a 3x3 design (3 distances from the application source and three replicates within the same distance) with cages containing 25-30 females each. After Permanone® application, dead and surviving females were counted and collected for genomic DNA isolation and molecular *kdr* mutations identification in the Na$_v$ channel. These analyses were performed either by PCR followed by agarose electrophoresis and/or sequencing (*Cx. quinquefasciatus*), or by melting curve analyses (*Ae. aegypti*). High frequency of *kdr* mutations was observed in populations of both mosquito species. In *Cx. quinquefasciatus* the *kdr* mutation (L982F; TTA to TTT) in the Na$_v$ channel is highly frequent with 81.2% of females being homozygous resistant. In *Ae. aegypti*, both the V1016I and F1534C pyrethroid resistant genotypes were widely distributed and at high frequency, with 77% of the females being double homozygous resistant (II/CC), this being the first report of *kdr* mutations in *Ae. aegypti* in Harris County. Linear regression models were utilized for both species and while the models detected the "operational control area of origin of mosquitoes", "distance from the application source", and "cage of mosquitoes within the same distance" as factors influencing survivorship, the *kdr* genotype(s) did not statistically affect probability of survival. Because Permanone® was applied in the field at the maximal allowable rate, we concluded that mutations in the sodium channel do not significantly affect effectiveness of control, while other environmental and operational factors contributed to the variability in survivorship observed. For *Cx. quinquefasciatus*, however, susceptible field mosquitoes could not be included in the model due to their insignificant numbers, and this may affect the interpretation of the model results. Further discussion will be presented.

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Tracking down invasions of the yellow fever mosquito, *Aedes aegypti*, using molecular markers

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The yellow fever mosquito *Aedes aegypti*, invaded the American continent from Africa during European trips of colonization approximately 500 years ago, and made its way to Europe and Asia in the 1800's. The species is now well adapted to humans and is the major vector of arboviral diseases like Zika, dengue, chikungunya, and yellow fever viruses worldwide.

In the last decade, we have genotyped *Ae. aegypti* populations throughout the world using 12 microsatellite loci and ~25,000 genome-wide single nucleotide polymorphisms (SNPs), creating an extensive global database that can be used to investigate historical and recent invasion events. These genetic markers have proven useful to study demographic parameters such as population structure, temporal stability, and bottleneck events in *Ae. aegypti* populations.

Our work has shown that *Ae. aegypti* diverged from its nearest described relative *Ae. mascarensis* approximately 7 million years ago and moved into Africa approximately 85,000 years ago. We find that the two recognized subspecies of *Ae. aegypti*, *Ae. aegypti formosus* (Aaf) a sylvatic darker form confined to the African continent and *Ae. aegypti aegypti* (Aaa) a domesticated lighter form that occur predominantly outside Africa, are genetically distinct and diverged less than 1,000 YA.

We have also used these markers to build a reference panel to determine the origin of new introductions of *Ae. aegypti* to the America's and Europe. Our work highlights the importance of having a comprehensive genetic reference panel in place for invasive species. We emphasize the need for a close collaboration between vector control and public health departments with genetic laboratories to achieve an effective, efficient, and sustainable control of invasive mosquitoes, such as *Ae. aegypti.*

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Addressing vector-borne diseases from communication, advocacy, and rights perspective approaches: the example of Chagas

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Chagas is a complex socio-environmental health problem characterised by the dynamic interrelation of different aspects with dimensions of diverse nature (biomedical, epidemiological, sociocultural, political-economic, and others). In other words, Chagas is much more than a disease and, in order to address it, it is essential to understand not only key aspects of the parasite, its routes of transmission, and medical aspects, but also the views of the different actors involved, public policies, the rights of the affected people... Admitting this helps us understand that any answer that seeks to generate contextualised and sustained solutions over time, must include and bring these different dimensions into play. Furthermore, it situates Chagas as a paradigmatic example when thinking about other health problems in general, and other vector borne diseases in particular. In this talk, rather than theoretical elements and conclusions, I will share questions and actions that I hope will later contribute to reach a collective conclusion on the roles that communication, academic work, participation, and social mobilisation have, in materialising concrete steps with political impact from a rights perspective. The "behind the scenes" of World Chagas Disease Day, the recent regulation of the Argentine National Chagas Law, some transdisciplinary actions in public communication, and collaborative work for progressing in crucial aspects regarding the visibility and respect of the rights of people affected by this problem, are all eloquent examples showing the multidimensionality that should guide our steps in the future. A few decades ago, Carlos Morel (a Brazilian researcher dedicated to Chagas) said that we have no choice but to be imaginative, flexible, and unprejudiced, when thinking about new research agendas on the subject. We believe that his words are more contemporary than ever, challenging us not only in reference to new research agendas but - fundamentally - in recognising our political role as beads of a kaleidoscope where diverse types of knowledge, scenarios and social actors come into play.

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Juvenile hormone shapes vector ecology

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Juvenile hormones (JHs) are insect-specific sesquiterpenoids that play vital functions regulating development, metamorphosis, reproduction, diapause, behavior and polyphenism. JH performs important roles in almost every aspect of vector ecology, including host seeking, feeding and mating behaviors, as well as reproductive output. Their importance lead to the development of JH analogs to be used as insecticide to control insect pests and vector of diseases. In the first part of the talk, I will review recent advances in our understanding of JH biology; including JH detection, regulation of hormone biosynthesis, JH transport and metabolism, hormone signaling and hormone actions. Afterward, we will discuss JH-regulated physiological aspects of insect life that provide a context for a better understanding of how JH influences the ecology of vectors.

JH impacts different life-history traits, including mosquito swarming, mating and oviposition behaviors; therefore affecting population growth and disease transmission. There is also evidence that JH might influence viral infection and pathogen transmission. We will also discuss what questions remain unanswered about JH biology and the roles of JH shaping vector ecology.

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SYMPOSIA

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Symposium "Vector control and eco-epidemiology of Chagas´s disease"

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This symposium focuses on vector biology, pathogen-vector interaction and eco-epidemiology of Chagas disease, the most important zoonosis in terms of the public health and the economic impact in Latin America. The expert panelists will present their studies, discuss issues and make recommendations for a certain course of action on: relationship between domestic invasion by native triatomines and habitat fragmentation; difference between domesticated and non-domesticated species, the case of *Triatoma dimidiata;* fluralaner treatment of dogs as a potential approach to be used to control vector transmission of *Trypanosoma cruzi* in pyrethroid-resistant populations of *Triatoma infestans*; what else we can learn about the transmission dynamics of *T. cruzi* in the domestic cycle in Chaco communities; evidences of the importance of an ecohealth approach for long term surveillance and control of Chagas in rural areas. It is an opportunity to think critically about new issues, engaging in constructive debate, and sharing new skills to address common concerns on this neglected disease.

Coordinators:
CÉCERE, Carla
ROJAS de ARIAS, Antonieta

Speakers:
ACOSTA, Nidia
CARDINAL, Marta V
MONROY, Carlota
WEINBERG, Diego
CARDOZO, Miriam

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Transmission dynamics of *Trypanosoma cruzi* discrete typing units in the Paraguayan Chaco communities. Natural hosts and vectors involved

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Active *Trypanosoma cruzi* transmission persists in the Gran Chaco region, which is considered hyperendemic for Chagas disease. This parasite is characterised by high levels of genetic heterogeneity, currently being grouped into six phylogenetically discrete typing units (DTUs TcI to TcVI), establishing complex domestic and sylvatic cycles. In the Paraguayan Chaco communities five of these six groups have been reported, circulating sympatrically. DTUs identified in the domestic area, in several surveys, included: TcI in *T. infestans* and *T. sordida* (mostly) specimens, whereas TcII, TcIII, TcV and TcVI were isolated from humans, dogs and *T. infestans*. Only the DTU TcIII was present in the sylvatic environment, in armadillos from three species, encompassing *D. novemcinctus*, *E. sexcinctus* and *Chaetophractus* spp. The high genotype resolution achieved with the combination of sequencing two single copy nuclear genes: Ascorbate-dependent haemoperoxidase (Tc*APX*) and Galactonolactone oxidase (Tc*GAL*), and by 15 variable microsatellite loci, let to the identification of twenty-five different multilocus genotypes (MLGs) from forty four isolates of *T. cruzi,* scattered among indigenous communities. Based on this, three main preliminary observations can be highlighted of the dynamics *T. cruzi* transmission in the domestic cycle. Firstly, the rapid and clonal propagation of the parasite, mediated by the insect vectors and/or human movements, demonstrated by the presence of identical MLGs in distant localities. Secondly, the finding of isolates with diverse MLGs in the same locality, that could indicate separate routes of introduction and/or dispersion or changes introduced in the parasite genome after the propagation (microevolution). Finally, it was possible to observe subpopulations among parasites belonging to the same DTU. Thus, the TcII genotype present in two localities had differences with respect to the TcIIs dispersed in the other communities. The microsatellite locus (vicp2b) enabled the discrimination of two TcVI subpopulations. On the other hand, the sylvatic cycle showed different transmission patterns with respect to the domestic, not compatible with rapid host or vector dispersal, with the finding of seven distinct sylvatic TcIII MLGs present in the same locality. Haplotype resolution of the two nuclear sequenced genes allowed observing the same haplotype in isolates from the domestic and sylvatic environments, supporting a certain level of connectivity between both cycles. Control and surveillance measures must be continuous and sustainable over the time in this area, since there is a constant risk of introduction of the parasite and the vectors from the sylvatic environment, and then, a rapid dispersal among communities may occur.

Control of pyrethroid-resistant *Triatoma infestans* by treating dogs with fluralaner in the Argentine Chaco

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Pyrethroid-resistant *Triatoma infestans* populations are widespread in the Argentine Chaco. They pose severe obstacles to ongoing elimination efforts. We conducted a placebo-controlled before-and-after efficacy trial in 28 infested sites in Castelli (Argentine Chaco) over 22 months. All 72 dogs initially present received either an oral dose of fluralaner (treated group) or placebo (control group) at month 0 posttreatment (MPT). Preliminary results justified treating all 38 control-house dogs with fluralaner 1 month later, and 71 of 78 existing dogs at 7 MPT. Site-level infestation and triatomine abundance were evaluated using timed manual searches with a dislodging aerosol. Vector infection was mainly determined by kDNA-PCR and blood meal sources by ELISA. In the fluralaner-treated group, infestation dropped significantly from 100% at baseline to 18-19% over 6–22 MPT whereas mean abundance fell highly significantly from 5.5 to 0.6 triatomines per unit effort. In the placebo group, infestation dropped similarly post-treatment. The overall prevalence of *T. cruzi* infection steadily decreased from 13.8% at 0–1 MPT (baseline) to 6.4–2.3% thereafter while in domiciles, kitchens and storerooms it dropped from 17.4% to 4.7–3.3%. Most infected triatomines occurred in domiciles and were human-fed. Infected-bug abundance plummeted after fluralaner treatment and remained marginal or nil thereafter. The human blood index of triatomines collected in domiciles, kitchens and storerooms highly significantly fell from 42.9% at baseline to 5.3–9.1% over 6–10 MPT, to increase to 36.8% at 22 MPT. Dog blood meals occurred before fluralaner administration only. The cat blood index increased from 9.9% at baseline to 57.9–72.7% over 6–10 MPT and dropped to 5.3% at 22 MPT, whereas chicken blood meals rose from 39.6% to 63.2–88.6%. Fluralaner severely impacted infestation and transmission-related indices over nearly two years, causing evident effects at 1 MPT, and deserves larger efficacy trials.

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Control of Chagas disease in Central America, the difference between domesticated and non-domesticated species, the case of *Triatoma dimidiata.*

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Rhodnius prolixus and *Triatoma dimidiata* were the main vectors of Chagas disease in Central America (C.A). *R prolixus* was introduced in C.A, thus it was strictly domesticated, but our native species could be found in domestic, peri-domestic and wild environments, it is a species with seasonal movements and is a complex of species. Since the years 2000 a massive insecticide campaign was performed in several C.A countries; resulting in the elimination of *R. prolixus*, but in the case of *T. dimidiata* the species did not disappear and fast re-infestation still very frequent. New control strategy based on risk factors for the presence of *T dimidata* inside mud houses were implemented. Local materials and community participation were used to improve the cracks and crevices on the mud walls, the dirt floor upgrading and management of animal houses were applied. Evaluation of the interventions were done using several techniques to assed the triatomine blood meals to appraise if human blood presence diminishes. Entomological evaluations were performed in short, intermediate and long terms. The long term evaluation after 16 years showed a very low infestation presence (below the transmission level) and also 80% of the houses maintained the walls without cracks and crevices. High spots areas of transmission (with acute cases) were addressed with the house improvements and chicken's coops implementation in more than 32 villages in the border with El Salvador; realizing also community surveillance with boxes and community volunteers. Community satisfactions of the house improvements were also assed. The new strategy for *T. dimidiata* seems to be usefully to diminish transmissions of the disease, improving the Life-quality of the communities motivating the prosperity of communities towards the Sustainable Development Goals by the United Nations.

Control of the vector of Chagas disease in the rural context of the Argentine Chaco

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Chagas Disease (ChD) is endemic en Argentina and, after the implementation of a national control program by the Ministry of Health since 1962, there are still an estimated 1.6 million individuals infected. Traditional control programs were based almost exclusively on entomological surveillance and chemical control of households but they were not continuous in their implementation due to a lack of coordination and both human and material resources. Specifically, in Argentina, the program was originally vertical and centralized and later partially transferred to the provinces with more or less success, and with many implementation difficulties in the area included in this study, the province of Santiago del Estero. Herein, the implementation of an ecohealth control program in rural settlements is described. This program started in 2005 in the city of Añatuya, Santiago del Estero, and is based on entomological surveillance and control, with the incorporation of health promotion and sanitary house improvement to make them not only refractory to infestation, but also to improve general living conditions. This approach, together with the establishment of social networks and community participation is still ongoing, and up to 2019, incorporated 13 settlements and 502 households. During the surveillance phase, 4,193 domiciliary inspections were performed and a reduction in both the intra- and peri-infestation rate from 17.9% to 0.2% and from 20.4% to 3%, respectively, was achieved. This program is ongoing and being implemented continuously for more than 14 years with high participation and adherence due to the building of social networks. Moreover, it has shown to be effective for long term surveillance and control of ChD, evidencing the importance of an ecohealth approach, which has not only enabled access to diagnosis and treatment for the community with minimal risk of re-infection, but has also improved the living conditions of those involved.

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Does environmental fragmentation affect the invasion of rural dwellings by triatomines?

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As the relative epidemiological importance of *T. infestans* decreased because of the success of vector control programs in South America, native populations of these species, as well as other secondary vectors of Chagas Disease attracted more attention. In the last two decades, an increasing number of studies reported the occurrence of sylvatic triatomines dispersing actively to domestic environments in the Cerrado and Atlantic Forest Regions of Brazil and in the dry western Chaco Region of Paraguay and Argentina. Anthropic modification of the landscape is mentioned as one of the main determinants of domestic invasion. The association between sylvatic populations of triatomines and habitat fragmentation is indirect and it is related mainly to the negative effect of environmental disturbance on the food source and wild refuge availability for sylvatic triatomines. Considering that the active dispersal of triatomines is related to the search for food and refuge, we hypothesized that domestic invasion by native triatomines will be more frequent in disturbed environments, as a consequence of habitat fragmentation. The aim of this study was to describe the occurrence and frequency of sylvatic triatomines invading rural houses, and to evaluate the effect of habitat fragmentation and other ecological factors on the invasion of rural houses in central Argentina. The entomological data was collected by community-based vector surveillance during fieldwork carried out between 2017–2020, over 131 houses located in fourteen rural communities in the northwest of Córdoba Province (central Argentina). Using a multi-model inference approach and generalized linear models we evaluate the effect of (i) the environmental anthropic disturbance in the study area (considering three categories: preserved, intermediate and disturbed landscape), (ii) the composition and configuration of the landscape surrounding the house at microscale (iii) the spatial arrangement of houses, (iv) and the availability of artificial refuges and domestic animals in the peridomicile, on the occurrence and frequency of house invasion by triatomines. We report the occurrence of seven native species of triatomines invading rural houses in the study area -*T. infestans*, *T. guasayana*, *T. garciabesi*, *T. platensis*, *T. delpontei*, *T. breyeri* and *P. guentheri*-. Study data show that invasion by triatomines occurs with higher frequency in disturbed landscapes, with houses spatially isolated and in proximity to subdivided fragments of forest-shrub cover. These results suggest that even under suboptimal conditions (loss of wild hosts and potential refuges), heavily disturbed environments support higher indices of triatomines dispersal. In conclusion, since these sylvatic species are not subject to chemical control by the control vector programs, the approach to the domestic environment represents a potential epidemiological risk. Therefore, study of the ecological, physiological and behavioral factors that modulate the active dispersal of sylvatic triatomines to the domestic environment deserves more attention.

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Symposium "Mosquitoes and ecoepidemiology"

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This symposium presents some advances in research on the ecoepidemiology of mosquitoes in Latin America, with a special emphasis on *Aedes aegypti*, given the magnitude of recent dengue (among other arboviruses) epidemics. First, Manteca Acosta will summarise integrated actions carried out by an interdisciplinary team from diverse institutions in Corrientes province, Argentina, in response to yellow fever (YF) epizootic events in the bordering southeastern Brazil. The remaining populations of howler monkeys were surveyed, as well as the YF mosquito vector fauna sharing their habitat in wild and rural environments. *Aedes aegypti* and *Haemagogus leucocelaenus* were the main vectors detected. Work was done on the update and reinforcement of the early warning community network against YF outbreaks using howler monkey groups presence as YF sentinels. Next, Cevallos focuses on *Aedes* mosquitoes, investigating household factors and/or characteristics associated with the mosquito density in two Pacific coastal cities, Guayaquil and Manta, aiming to identify the relationship of key factors that can help to optimize vector control programs. Overall, a major role was detected of variables associated with water and waste management and humidity, supporting that access to municipal services was effective at reducing mosquito proliferation in households. Container -level characteristics correlated with *Aedes aegypti* pupae counts are discussed. Evidence is presented supporting the role of education in reducing mosquito populations. Afterwards, Secundino focuses on vector-virus interactions, by investigating how multiple infections with combinations of strains of CHIKV, DENV, ZIKV, and YFV that are currently circulating in Brazil, affect vector competence, infection rate, viral abundance, and the survival of a Brazilian *Ae. aegypti* population. Finally, *Ae. aegypti* has recently expanded its distribution in South America towards colder climate regions. Fischer addresses hypotheses regarding possible adaptations of *Aedes aegypti* to colder climate regions, linked to increased tolerance to low temperatures and to a better adjusted hatching response to environmental conditions, which will likely result in further range expansions.

Coordinators:
GLEISER, Raquel M
BRAGA, Ima A
PIMENTA, Paulo FP
SCHWEIGMANN, Nicolás J

Speakers:
MANTECA ACOSTA, Mariana
CEVALLOS, Varsovia E
SECUNDINO, Nagila FC
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Eco-epidemiology of Yellow Fever in the Northeast of Argentina

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During 2020-2021 there were several yellow fever (YF) epizootic events in southern Brazil, close to the Argentina border. Corrientes province, which limits to the east with Brazil, lacked a plan to take actions when YF howler monkey cases were reported in Brazil, then we decided to carry out integrated actions considering all scientific-technical components regarding to this disease. We put together an interdisciplinary team including the CeNDIE-ANLIS Malbrán, EBCo-CECOAL-CONICET, CEPAVE-CONICET, IUCS Fundación H.A. Barceló, and the Directorate of Control of Diseases Transmitted by Vectors. The following actions were carried out: 1) surveys and monitoring of the remaining populations of black and gold howler monkeys (*Alouatta caraya*) to verify their presence/absence in areas where they suffered a YF outbreak in 2009; 2) update of the early warning community network against YF outbreaks using howler monkey groups presence as YF sentinels, 3) YF vector sampling in wild and rural environments to determine the specific composition of culicids that share habitat with howler monkeys. A team of 10 people visited several sites including forests from April 5 to 10, 2021. Although groups of monkeys or evidence of them were found in most of the sites selected in this survey, their density was extremely low compared to the populations located along the Paraná River, confirming the critical situation of this species in NE Corrientes. We did reinforce, together with the Vector Directorate of the Province of Corrientes, the early warning community network, identifying and adding new referents, and re-linking with those previously contacted. Regarding the mosquito community, 18 species of 8 different genera were collected. Among them, *Aedes aegypti* and *Haemagogus leucocelaenus* are main vectors in America of the YF virus in the urban and jungle cycle, respectively. Other species involved or potentially involved in the jungle cycle were: *Sabethes albiprivus*, *Sa. identicus*, *Ae. scapularis*, *Ae. serratus*, *Mansonia indubitans*, *Psorophora albipes*, and *Ps. ferox*. We suggest the promotion of long-term monitoring of groups of howlers in the area and mosquito vectors in their roosts, the formalization of the early warning community network, implementing non-formal education workshops in schools and similar institutions to call attention to the situation of black and gold howler monkeys, their role as sentinels of public health, and the importance of maintaining healthy ecosystems.

Household risk factors for the abundance of Aedes aegypti in Ecuador

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Vector-borne diseases are a major cause of disease burden in Ecuador, especially arboviruses spread by *Aedes aegypti* mosquitoes. Household characteristics are one of the main disease risk factors. We investigate the proximal determinants of Aedes mosquito density in two Pacific coastal cities, Guayaquil and Manta, to identify the relationship of key factors that can help to optimize vector control programs. Cross-sectional entomological surveys were conducted in Guayaquil city between 2013 and 2016 and Manta between 2018 and 2020. In Guayaquil we surveyed 610 households, we included household demographics, municipal services, potential breeding containers, presence of *Ae. aegypti* larvae and pupae, and records of mosquito control. A zero-truncated negative binomial regression model was fitted to data for estimating the household pupal index. An additional model assessed potential household-level risk factors for *Ae. aegypti* pupal proliferation. In Manta city we defined two types of neighborhoods namely high and low with at least 96 households, sampled eight households in each of the four neighborhoods monthly for 12 months and collected survey data and sampled mosquito populations. In Guayaquil, bed canopy use, unemployment, container water volume, and the interaction between large solid collection and rainfall 2 weeks before the sampling event were positively correlated, with the strongest effects from large solid collection and bed canopy use. Stagnant water is positively correlated with Ae. aegypti pupae counts while breeding sites composed of car parts, furniture, sewerage parts, and vases, were all negatively correlated. In Manta, we collected a total of 740 mosquitoes. The Prokopack aspirators yielded the largest number of collected mosquitoes at each site compared to BG-Sentinel2 traps, CDC miniature light traps and CDC resting traps. The survey indicated that water management and household characteristics directly affect mosquito population abundance. Access to municipal services in Guayaquil was effective at reducing mosquito proliferation in households. Association of bed canopy use with higher mosquito densities is unexpected and may be a consequence of large local mosquito populations at night (*Culex* spp.). The impact of rainfall on mosquito density is multifaceted, as it may both create new habitat and "wash out" existing habitat. In Manta, meteorological, climatic, and socioeconomic variation have all likely contributed to shaping conditions conducive to *Aedes* density across all the neighborhoods. Wealthier households, where occupants displayed higher knowledge of arboviruses and the absence of structural points of entry for mosquitoes, were associated with lower *Aedes* density. Analyses of households in Manta suggested a major role of variables associated with water and waste management and humidity. Interestingly, using pesticides in Manta, and using bed nets across study sites showed a positive association with *Aedes* density. Wealthier households may also contribute to *Aedes* breeding, potentially through the landscape and vegetation elements.

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Brazilian *Aedes aegypti* as a competent vector for multiple complex arboviral coinfections

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Several arboviruses are transmitted by *Ae. Aegypti* to humans, such as Chikungunya virus (CHIKV), dengue virus (DENV), Zika virus (ZIKV) and yellow fever virus (YFV), are in simultaneous circulation in tropical countries. This mosquito is widespread in many countries of the New World, primarily throughout urban areas of Latin America, is highly competent for the aforementioned four arboviruses, and has the potential to cause coinfections in humans. Humans may acquire coinfections by either being bitten by different mosquitoes, each infective with a distinct arbovirus or bitten by a single vector mosquito simultaneously infective for multiple arboviruses. The public health threat posed by coinfected vectors is not well understood and greatly underestimated. Herein, we investigated the dynamics of multiple complex infections with combinations of CHIKV, DENV, ZIKV, and YFV using the strains that are currently circulating in Brazil to understand how multiple coinfections affect vector competence, infection rate, viral abundance, and the survival of a Brazilian *Ae. Aegypti* population obtained in a disease-endemic city. The *Ae. aegypti* mosquitoes were infected or coinfected with circulating strains of chikungunya, dengue, Zika, and yellow fever in 15 combinations. The infection rate, viral abundance, vector competence, disseminated infection rate, and survival rate after the experimental infections were recorded. Infection rates were 100% in all single and multiple infection experiments, except in one triple coinfection that presented a rate of 50%. The vector competence and disseminated infection rate varied from 100% (in single and quadruple infections) to 40% (in dual and triple infections). The dual and triple coinfections altered the vector competence and/or viral abundance of at least one arboviruses. The highest viral abundances were detected for a single infection with chikungunya. The viral abundances in quadruple infections were similar when compared to each respective single infection. A decrease in survival rates was observed in a few combinations. In conclusion *Ae. Aegypti* from a Brazilian city with a recurrent circulation of ZIKV, DENV, YFV, and CHIKV was able to host all the four viruses in single and multiple complex coinfections, which demonstrates a severe risk of simultaneous arbovirus transmission to humans. Also, a similar pattern of parameters for all viruses in single and quadruple infections, in contrast to dual and triple ones, suggests the mmune response of *Ae. aegypti* to viral infection acts differently depending on the number of conditions. Finally, the mosquito's immune response depends on which arbovirus species and family are involved in the multiple infections, as seen by CHIKV infection in combination with the other viruses.

Is *Aedes aegypti* adapting to colder climates? Implications for future range expansion

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The mosquito *Aedes aegypti*, vector of dengue and other arboviruses, has recently expanded its distribution in Argentina towards colder climate regions, where low winter temperatures limit the development and reproduction of this species. This observed range expansion might be favored by adaptations of the populations to the thermal conditions of the recently colonized environments. In this presentation we will address two hypotheses regarding possible adaptations of *Aedes aegypti* to colder climate regions: 1) populations from temperate regions have an increased tolerance of immature stages to low temperatures, which might favor that individuals complete their development (and eventually reproduce) during an extended period of the year, avoiding an excessive mortality at the beginning and the end of the cold season; 2) populations from temperate regions have a better adjusted hatching response to environmental conditions like low temperatures and short photoperiods, which allows for a reduction in larval mortality due to untimely hatching of the eggs. Different studies performed during the last decade with Aedes aegypti from Argentina assessing these two hypotheses will be presented and discussed. The results provide evidence of: 1) an ongoing adaptation of immature stages to lower temperatures; 2) an ongoing adaptation of the hatching response at low temperatures; and 3) the induction of embryonic diapause in response to short photoperiods (observed for the first time in this species). These findings might explain the expansion and successful persistence of *Aedes aegypti* in increasingly colder areas of Argentina, and further range expansions of this species should be expected.

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Symposium "Mosquitoes control status and challenges"

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This symposium presents some challenges and improvements regarding mosquito surveillance and control actions in Latin America, mainly focused on *Aedes aegypti*. In Uruguay, different methodologies have been used to monitor *Aedes aegypti* since its detection in 1997, mainly based on larval surveys. Willat summarises improvements made in Uruguay of the *Aedes aegypti* surveillance system based on an ovitrap grid, aimed at better identifying sites and periods with high *Aedes* density to better focus prevention activities and control measures, to help prevent future outbreaks of arbovirosis. The Covid 19 pandemic diverted the attention and participation of the entities in charge of controlling diseases transmitted by *Aedes aegypti*, which surely influenced the significant increase in the number of dengue cases. Velasquez shares a descriptive study developed in Ecuador with medical students from the University of Guayaquil to establish, on the one hand, social determinants of health and control strategies reported by the community during the SARS-CoV epidemic, and to estimate types of larval habitat and the abundance of immature stages of the vector in student residences. The importance of continued community participation strategies and their involvement in the design of their own control strategies is discussed. To achieve the reduction and/or elimination of vector-borne diseases, apart from community participation, a close link between academia, health professionals, and policymakers is important. Pereira reports on the implementation of surveillance with ovitraps in several municipalities of Brazil to assist in vector control and to evaluate the effectiveness of the control strategies used. The implementation of this methodology in the country is led by Laficave/IOC/Fiocruz in partnership with the General Coordination of Arbovirus Surveillance CGARB/MS, aimed at strengthening entomological surveillance. It is widely accepted that scientific knowledge is important in the decision-making process for vector borne diseases. For example, the development of epidemiological models seeks to predict the places with the highest risk of transmission of mosquito-borne diseases, or the effects of implementing some management strategy. Solari will take us down a different avenue by discussing whether there is just one science, or if there are several possible sciences, and what this philosophical query has to do with SOVE and vector control.

Coordinators:
GLEISER, Raquel M
BRAGA, Ima A
PIMENTA, Paulo FP
SCHWEIGMANN, Nicolás J

Speakers:
WILLAT, G
VELASQUEZ SERRA, Glenda C
LIMA, José BP
SOLARI, Hernán GI

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Surveillance and control of *Aedes aegypti* in Uruguay, achievements and challenges

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Uruguay has monitored the presence of *Aedes aegypti* since 1997, when its reintroduction was reported. Since then, different methodologies have been used (larval surveys, control of strategic points, method of rapid survey of *Ae. aegypti* indices, traps). The LIRAa methodology is effective in detecting the main containers that are acting as breeding sites for *Ae. aegypti* in a certain area. However, the larval indices obtained are not sufficient to estimate the risk of arbovirus transmission because they do not provide a reliable estimate of the adult population, they do not take into account the distribution in transmission clusters and are heavily operator-dependant. The objective of this work was to improve the surveillance system in order to identify sites and periods with high *Aedes* density and help prevent future outbreaks of arbovirosis. Following the advice from PAHO/WHO, we installed a net of ovitraps 400 meters apart, covering the entire area of the locality under surveillance. Initially, three pilot cities were selected and more were incorporated gradually. An instruction manual was prepared to guide the local referents in the surveillance process. Traps were installed and checked weekly, counting the *Aedes* eggs with a stereomicroscope. The number of eggs on each trap was registered using an electronic spreadsheet. In order to evaluate the temporal variation of *Aedes*, two indices were calculated weekly: the percentage of positive ovitraps, an approximation of the distribution of the vector in a locality; and the mean of eggs per ovitrap, related to the vector density. Spatial analysis was performed using a geographical information system. Each trap was georeferenced, and heatmaps were developed using the data obtained in each point. In order to make the data more accessible and easy to view and share, a computer visualization tool was developed. Temporal variation of entomological indices was described in each locality, allowing comparison between years and sites. Control and prevention measures were recommended for every stage of the seasonal variation (no activity, oviposition beginning and increase, peak). The spatial analysis allowed to detect hotspots of *Aedes* density in every locality. Moreover, the most important hotspots were conserved between years, despite the lack of vector activity in the winter months. Control activities (breeding sites detection and elimination, education) were planned in the spots of higher *Aedes* densities. The ovitrap surveillance system provided reliable entomological information that can be used in the design of control and prevention activities. Knowing the seasonal variation of the vector allowed focusing prevention activities in the period of low vector activity and the control measures in the peaks of activity. Mapping the sites of higher vector densities optimized the field work by limiting the interventions to a few sites in each city. Relations of entomological indices and density hotspots with arboviral outbreaks should be evaluated in the future.

Control strategies for the *Aedes aegypti* vector in times of SARS-CoV-2

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During the Covid 19 pandemic *Aedes aegypti* borne diseases such as Dengue cases increased exponentially due to the little or almost null participation of the entities responsible for control. For this reason, health systems of endemic countries to these tropical diseases must create strategies to reduce mortality from these pathologies. This study aimed to establish social determinants of health in the sampled analysis units, estimate the type of breeding site, abundance of immature stages of the vector, and identify control strategies reported by the inhabitants of the community during the SARS-CoV epidemic. This is a descriptive, field, non-experimental and cross-sectional study, carried out from March 12 to April 12, 2021 in Guayas Province, Canton Guayaquil, Ecuador. To do this, due to the pandemic situation, it was requested the site of residence of all the students from the fifth and sixth semester of Medicine career, of the University of Guayaquil. The type of sampling was selected by conglomerate in a single stage. In each dwelling was interviewed the head of family, with prior informed consent in a virtual platform (Zoom UG). Also, a structured instrument was applied, containing in one of the sections, charts with drawings related to stages of the mosquito. Population were trained in the recognition of different barriers and strategies for the control of larvae and adults in selected areas. Summary measures, like percentage, dispersion (mean) and the statistical indicator $\chi 2$ not corrected and Mc Nemar with level of significance $\alpha = 0.05$ were used. 247 surveys were carried out. The X of the interviewed population corresponded to the range of 25-35 years. The environmental determinant was identified more frequently. Temperature ranged between (28-31 ºC), relative humidity (99%) and an accumulated monthly rainfall of 200.5 mm. 78.4% of homes have vegetation in the surroundings. 43% of the residents had contracted some serotype of the dengue virus and close to 73.2%, COVID 19. At least one inhabitant per family concurred with both pathologies. Coincidentally, between 1-3 of its members had associated comorbidities (e.g., diabetes, high blood pressure and asthma). 39% of those interviewed do not have health services. Low containers were the most frequent breeding place. L2 was the most identified stage. 11.5% of the residents cover the containers where they collect water. During the pandemic, population was not warned about prevention of dengue. Regarding the knowledge to eliminate mosquito breeding sites, 79.2% responded appropriately. However, they consider the following strategies in the following order: use of repellents, burning of medicinal plants, breeding sites removal. It is necessary to continue leading community participation strategies that try to understand the relationship between the focal environmental characteristics with the presence of the vector, in order to design their own strategies considering the new scenarios, challenges and diverse geographical contexts that the *Aedes* Control Program must face in the Americas.

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Entomological Surveillance - A challenge for vector control programs

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The most adopted practices to prevent the proliferation of vector mosquitoes in urban areas is the engagement of the population in preventive practices and in the mechanical control of larval breeding sites. Additionally, the use of insecticides continues to be a fundamental strategy to complement vector control, in its various aspects, such as application of larvicides in breeding sites, residual spray in homes and strategic points, spatial application, and the use of impregnated mosquito nets and screens. However, the continuous and often exaggerated use of these chemicals has led to the selection of resistance of vector populations to the compounds used, significantly interfering in vector control programs. Thus, entomological surveillance becomes essential both to indicate the best tools to be used according to local needs, to indicate the most suitable product for a given population of mosquitoes, as well as to evaluate the effectiveness of the tools that are being used. In this sense, the implementation of surveillance with traps is importance to assist in vector control and in this context, the ovitrap emerged as an important tool due to its low cost and ease of handling. Aiming at strengthening entomological surveillance in Brazil, Laficave/IOC/Fiocruz, in partnership with the General Coordination of Arbovirus Surveillance CGARB/MS, has been working to implement this methodology in the country. We believe that the surveillance implemented in the municipalities will contribute to the control of arboviruses in the country.

Controlling vectors: Do we need normal or post-normal science?

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Is there just one science, the one we have, the given? Or on the contrary, are there several possible sciences? In the latter case: which are their differences? Are they compatible? Complementary? Opposite? I will try to persuade the audience that at least three sciences can be distinguished: original blend, techno-science (normal science) and science of the postnormal age (call it transcendent science as it needs to go outside its field into the realm of decision making). I will distinguish the three at the epistemological level (arguably the deepest level) so that it may happen that what is scientific knowledge for one is not so for another. Normal science is mostly concerned with the development of technologies, in turn, the transcendent science must meet challenges outside its own realm, such as: global warming, global epidemics policies, extinction of species, desertification, the nuclear energy use and in general all sort of interventions in the environment. What has to do this philosophical inquiring with SOVE? I will show that controlling vectors belongs to both normal science and science for the postnormal age and requires the collaboration of both forms. The actual problem being that transcendent science needs to be developed.

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Symposium “New perspectives and approaches for the surveillance and control of phlebotomine sand flies”

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Based on research developed in recent decades, it is known that the richness, diversity, abundance, populations, insect vector species complexes, reservoirs, and spatiotemporal dynamics, contribute to understanding the different transmission scenarios of cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). In this framework, research addressing leishmaniasis from different aspects such as eco-epidemiology, expansion, vector adaptation and new approaches for surveillance and control, among others, are important for generating appropriate prevention and control measures for each scenario. In this Symposium, experts on the subject were convened, taking gender equity and geographic representation into account, to discuss cross-cutting perspectives in integrated vector management, and to share research and progress being made in relation to CL and VL in the region.

Coordinators:	QUINTANA, María G VALDERRAMA CUMBRERA, Anayansi
Speakers:	MAIA-ELKHOURY, Ana NS VALDERRAMA CUMBRERA, Anayansi SALOMON, Oscar D PEÇANHA BRAZIL, Reginaldo

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Current epidemiological situation and leishmaniasis surveillance and control actions

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Leishmaniases are infectious diseases with a complex biological cycle that includes different species of parasites, reservoirs, and vectors, which can compromise the skin, mucosal, and viscera. In the Americas, leishmaniases are considered a public health problem due morbimortality and wide distribution. In 2019, the Pan American Health Organization (PAHO) Diseases Elimination Initiative was approved, with specific goals of controlling and eliminating leishmaniasis as a public health aligned with the WHO Roadmap 2021-2030. For cutaneous and mucosal leishmaniasis (CL/ML) the goal is to control, due to the difficulty in eliminating a zoonotic disease with mainly sylvatic transmission, with targets aimed at improving laboratory diagnosis in at least 90% of detected cases and reducing the proportion of children under 10 years old affected by the disease. For visceral leishmaniasis (VL), the goal is to reach elimination as a public health problem with targets aimed at reducing the incidence and lethality rate. In the Americas, CL is endemic in 18 countries and is the most frequent form. From 2001 to 2020, 1,067,759 CL/ML cases were reported with an annual average of 53,387 cases. In the last 20 years, there has been a decreasing trend in the number of cases, and in 2020 the lowest figure was reported (39,705) to the PAHO/WHO Regional Leishmaniasis Information System (SisLeish). The average case distribution is concentrated in the Andean Area with 41% of the cases, 40% in Brazil, 16.5% in Central America, and the rest in the Southern Cone, Non-Latin Caribbean, and Mexico. VL (caused by *L. infantum*) is the most severe form, as it can cause death in up to 90% of untreated people. It is endemic in 13 countries, and in 2001-2020, 67.845 VL cases were reported in the Region, with an annual average of 3.392 cases. 96% of cases were reported by Brazil, with an average of 3,268 cases per year, followed by Paraguay (57), Colombia (38), Venezuela (22), and Argentina (13). Bolivia, El Salvador, Guatemala, Honduras and Mexico have presented sporadic transmission over the years, and in 2018 the first human case was confirmed in Uruguay. From 2015 to 2020, despite a reduction in the number of cases, a geographic expansion has been observed. The proportion of HIV–VL coinfection cases increased, reaching 12.4% in 2020. The average VL fatality rate is 7%, but in 2020 it was 8.15% being three times higher than the global fatality rate. PAHO, through the Regional Program for Leishmaniasis, has supported endemic countries to strengthen surveillance and control actions, according to the epidemiological scenario, to reduce morbidity, mortality, and severe forms of the disease, increase access to early diagnosis, adequate and integrated treatment of cases, and implement actions to reduce contact between people and reservoirs with vectors of leishmaniasis.

Diversity and infection by *Wolbachia* and *Leishmania* in sand flies Diptera: Psychodidae) populations from different landscapes

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The Wolbachia: Rickettsiales bacterium that infects a wide variety of arthropods and is an endosymbiont within filarial nematodes, has the ability to manipulate host reproduction from different mechanisms. However, little is known about the infection rate of these endosymbionts in some species of sand flies that inhabit different biomes and the relationship with *Leishmania* species. In this sense, we made collections of sand flies in different types of habitat and most of the species were found in wooded habitats, but there are some species that were found in environments disturbed by deforestation. To determine if these species were infected with both *Leishmania* and *Wolbachia* strains depending on the habitat, we used the ITS-1 and spec primers, respectively. Our data show *Leishmania* or *Wolbachia* infection in some individuals, but the concomitance of both was also recorded and in a high proportion in the species captured in secondary forests. Although the rate of co-infection is low with respect to individuals infected only with *Leishmania* or *Wolbachia*, it is vital to understand the role of the relationship of both to build reliable strategies in order to decline the transmission of leishmaniasis and its impact as a neglected disease.

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Integrated surveillance of leishmaniases vectors in the Americas

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We developed integrated vector surveillance (IVS) proposals, both for cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) in the Americas, based on eco-epidemiological studies conducted by researchers of the Leishmaniasis Research Network of Argentina-REDILA. For CL, the transmission was explained in the framework of the edge effect, the increase of vectors and risk of exposure at ecotones and environmental interfaces, and typified as ephemeral, transient, or permanent edges. This approach supports a cost-effective IVS strategy for early warning of CL outbreaks, through an environmental modification alert network, which includes multiple sources of information and actors. In relation to VL, the earliest colonization sites and spatial distribution were explained by modeling and forecasting the most likely hotspots, persistent in time and space, and modulated by environmental variables. Therefore, for VL, a scalar strategy of critical site selection is proposed from a "city" scale based on secondary sources such as remote sensing for the definition of possible areas to monitor and intervene; a scale of restriction from possible areas to most likely areas through local knowledge; and a "focal site" scale of trap placement through field observation; in this way, IVS activities are carried out at a few sites of the urban landscape and allow a sustainable program. The VL surveillance strategy is being evaluated and validated in the field.

A new approach in the control of *Lutzomyia longipalpis* s.l. in Brazil

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Lutzomyia longipalpis (Diptera:Psychodidae) is the main vector of the protozoa Leishmania infantum the agent of American Visceral Leishmaniasis (AVL) and Brazil has the greatest incidence of this disease*. Lu. longipalpis* is the main vector and is considered to be a species complex. Different members of the complex produce different agregation/sex pheromones. In the last 20 years VL has increased it distribution to new areas in Brazil and current strategies for controlling sand flies using residual spraying have been unable to prevent the spread of the disease. We have now evidences showing that residual insecticides may interrupt "lekking" between males and females of *Lu. longipalpis* and provoke sand fly dispersion to new areas. However, the use of *Lu. longipalpis* synthetic sex pheromone (9-methyl-germacrene) plus residual insecticides may be a new control strategy in endemic areas and more long term field trials are needed to include this new approach as part of visceral leishmaniasis control program.

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Symposium "Tick-borne diseases (*Rickettsia*, *Ehrlichia*, *Borrelia*)"

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Ticks (Acari: Ixodida) are important vectors and transmit diverse pathogens, including protozoa, viruses, and bacteria. Tick-borne diseases can cause damage to both human health and domestic and wild animals. In the symposium, specialists will speak on the following topics related to tick-borne diseases with implications for public health in Latin America: Lyme disease in the area, focused mainly on its diagnosis; the status of relapsing fevers; the vectors of the spotted fever *Rickettsia rickettsii*; and about the possible vectors of *Borrelia burgdorferi*, *Ehrlichia* and *Anaplasma* to humans. The Latin American Society for Vector Ecology Congress, due to its wide and diverse audience and diversity of topics addressed, constitutes the ideal framework to discuss tick borne diseases in Latin America. In this sense, the development of the symposium will allow the participants to exchange knowledge and discuss future integrative projections and their importance.

Coordinators: LARESCHI, Marcela
VENZAL, José

Speakers: ARMITANO, Rita
VENZAL, José
TARRAGONA, Evelina L
MUÑOZ-LEAL, Sebastián

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Lyme disease in Argentina. Situation status

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Vector-borne diseases account for 17% of infectious diseases globally. Lyme Disease (LD) is caused by bacteria from the Borrelia burgdorferi Complex (B.b) sensu lato, which is transmitted to humans by the bite of ticks from the Ixodes ricinus Complex distributed in the northern hemisphere. To date, in Argentina, no autochthonous cases of LD caused by B.b have been reported, and no transmitting vector has been identified. Analyze through inquiries for suspicion of LD, received at the Special Bacteriology Service (SBS), INEI-ANLIS, the status of this disease in Argentina. During the period 09/2014-09/2017, 50 consultations were registered, from which 50 serum samples and 4 cerebro spinal fluid (CSF) samples were received. In the SBE, the detection of IgM/IgG antibodies was performed by Enzyme-linked immunosorbent assay (EIA) (IDEIA-OXOID), in serum and CSF, according to the manufacturer's instructions. 92% of the queries were from residents in Argentina, mainly in Autonomous City of Buenos Aires and the province of Buenos Aires (54%). The following data were obtained from the epidemiological form: 1) mean age, 44 years; 2) 54% were male; 3) 62% consulted for neurological symptoms and 15% for rheumatological symptoms; 4) 44% did not refer to a trip to an endemic area; 5) 46% reported tick bites; 6) 44% of the patients had never consulted a medical professional. Of the 50 patients studied, 9 had previous results (+) issued by private laboratories: 6/50 had Indirect Immunofluorescence (IFI) (+), one of these patients also had PCR (+); 1/50 presented EIA (+) and 2/50 showed EIA (+) and Western Blot (WB) (+). All CSF samples and 48/50 serum samples were non-reactive. Only two serum samples were reactive, these came from a patient with a trip to Poland and erythema migrans. In October 2017, 10 serum samples from 9 patients were sent to the CDC for IgM/IgG detection by EIA and WB. The selection criteria were: 1)- travel to an endemic area; 2)- results (+) by IFI, EIA or WB sent by other laboratories. 7/9 patients reported traveling to an endemic area and results (+) by IFI/EIA; the remaining two, despite not registering permanence in an endemic area, had EIA and WB results (+) issued by a foreign laboratory. The results obtained by the CDC were 100% consistent with those obtained in the SBE. Only 2/10 samples were reactive for IgM/IgG, the remaining 8/10 were non-reactive, this last group of samples included the sera with (+) results sent by the foreign laboratory. The field studies have not shown the presence of the vector in Argentina. Travel history and stay in a confirmed endemic area of LD are considered as inclusion criteria for diagnostic study. It is important to implement surveillance systems that consider local epidemiological characteristics and incorporate the use of diagnostic techniques recommended by the CDC reference laboratory that allow the diagnosis of imported cases to be resolved.

Possible vectors of *Borrelia burgdorferi*, *Ehrlichia* and *Anaplasma* to humans in Latin America

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Ticks (Acari: Ixodida) are hematophagous parasites that transmit a wide variety of pathogens to vertebrates, including viruses, protozoa, and bacteria (e.g. spirochetes and rickettsials), among others. The study of tick-borne diseases is highly developed in North America and Europe, although significant progress has been made in Latin America in recent years due to the use of techniques based mainly on molecular biology. Rickettsioses are the most relevant tick-borne diseases in public health in Latin America. *Rickettsia rickettsii* remains the most important pathogen, although rickettsioses caused by *Rickettsia parkeri sensu stricto*, *Rickettsia sp*. strain Atlantic Rainforest and *Rickettsia massiliae* are considered emerging diseases. On the other hand, Lyme disease is one of the tick-borne diseases that causes concern and confusion in the population. With the exception of Mexico, in Latin America the cases reported as autochthonous Lyme disease are clinical cases that have only Immunofluorescent Antibody test (IFAT), without confirmation by specific Western Blot (WB). In recent years, new genotypes of *Borrelia burgdorferi sensu lato* have been confirmed in ticks of the *Ixodes* genus in Latin America, although these Ixodes species do not frequently parasitize humans. For the *Ixodes ricinus* complex, *Borrelia burgdorferi* s.l. has been determined in South America in *Ixodes fuscipes* (as *I. pararicinus* and *I. aragaoi previously), I. pararicinus* and *I. cf. affinis*. Also, in other species of Ixodes in South and Central America, such as: *I. stilesi, I. sigelos, I. cf. neuquenensis, I. longiscutatus I. auritulus, and I. cf. boliviensis*. A similar situation occurs with the diagnoses of ehrlichiosis and anaplasmosis. With the exception of some cases confirmed by *Ehrlichia canis*, most of the reports in Latin America correspond to clinical diagnoses supported by basic serology without molecular confirmation. Strains closely related to *Ehrlichia chaffeensis* were detected in *Amblyomma spp.* and deer in Argentina and Brazil, but not associated with human clinical cases. Likewise, several strains of *Ehrlichia* that probably correspond to new species were also found in different species of ticks in the genera *Amblyomma, Haemaphysalis, and Ixodes*. In Latin American countries there is evidence of the circulation of one or more strains related to *Anaplasma phagocytophilum* in ticks, although the pathogenicity of these strains for humans is unknown.

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Vectors and potential vectors of *Rickettsia rickettsii*, causal agent of Rocky Mountain Spotted Fever in humans, for the Neotropic

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Rickettsia rickettsii is the causative agent of Rocky Mountain spotted fever (RMSF), the most severe rickettsiosis affecting humans in the Western Hemisphere. By contrast with other tick-borne rickettsia of the spotted fever group, *R. rickettsii* does not elicit an eschar at the tick bite site. Symptoms of the disease include high fever and headache that may be associated with malaise, myalgia, rash, nausea, vomiting, anorexia and diarrhoea. Additionally, RMSF may result in various neurological, pulmonary, renal and cardiac manifestations. In the Neotropic, cases of human rickettsiosis caused by *R. rickettsii* have been detected in Mexico, Costa Rica, Panama, Colombia, Brazil and Argentina, and current case fatality rates range from 20 to 40%. A tick-borne disease includes a triad consisting of: an arthropod vector, a susceptible vertebrate host, and a pathogen. For an arthropod to be considered a vector, it must have the competence to do so. The vector competence is defined as the intrinsic susceptibility of an arthropod species to become infected, replicate and transmit a pathogen. For the Neotropic, six species of hard tick vectors of *R. rickettsii* have been confirmed: *Amblyomma aureolatum, Amblyomma mixtum, Amblyomma patinoi, Amblyomma sculptum*, *Amblyomma tonelliae* and *Rhipicephalus sanguineus* s.l. The ecological parameters that significantly affect the cycle of a tick-borne zoonotic disease are: vector competition, seasonal activity of the vector, geographic distribution of the vector, host specificity, and habitat requirement. The status of a vector-borne disease can vary depending on the interaction between these five factors, that is, whether it behaves enzootic (relatively stable, with little or no change in disease incidence) or epizootic (unstable, with high increase in the incidence of the disease). So, the geographic distribution as well as knowledge of the seasonal activity patterns of a tick species is essential to determine the periods when people or domestic animals are at risk of acquiring infection from tick-borne pathogens. The aim of this dissertation is to make a brief review of the main neotropical tick species that transmit *R. rickettsii* and the ecological parameters that condition the life cycles of ticks species, such as geographic range distributions and seasonal activity.

What about tick-borne relapsing fever spirochetes in Latin America?

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Relapsing fever spirochetes (*Borrelia*) thrive in sylvatic foci switching infections between ticks and wild vertebrates. Humans are accidental hosts that get infected after the bite of infected ticks, while visiting caves or rocky environments where those ectoparasites occur. Soft ticks in the genus *Ornithodoros* are their main vectors. The current work summarizes the eco-epidemiological scenario regarding tick-borne relapsing fever spirochetes in Latin America. Tick-borne relapsing fever was once an important disease in Latin America with cases in Colombia, Mexico, Panama, and Venezuela. The etiological agent, *Borrelia venezuelensis*, was transmitted by *Ornithodoros rudis*, a soft tick that invaded human dwellings and also parasitized hen. Moreover, at least five species of soft ticks were implicated as vectors of spirochetes in Latin America before 1950. Thereafter, the disease was forgotten presumably because the attention was redirected to other vector-borne affections such as malaria. In recent years the rediscovery of *O. rudis* and the isolation of *B. venezuelensis* triggered again research regarding these neglected microorganisms in the Continent. Since then, novel *Borrelia* genotypes have been documented in *Ornithodoros hasei*, *Ornithodoros mimon*, *Ornithodoros tabajara*, and *Ornithodoros rietcorreai* in Brazil; *Borrelia puertoricensis* was isolated from *Ornithodoros puertoricensis* in Panama, and an array *Borrelia* spp. that still need extended biological and genetic characterization were detected in soft ticks from Chile and Colombia. It is evident now that tick-borne relapsing fever spirochetes are not gone in Latin America, and despite recent findings are promissory, diagnosis in humans still needs to be implemented. Indeed, we know that *Ornithodoros* spp. that harbor *Borrelia* spp. do bite humans, and cases of houses infested by these soft ticks are spread along the continent. Diagnosis capacity using darkfield microscope implies training technicians in hospitals in the areas where infected soft ticks occur. Moreover, tick-borne relapsing fever symptoms overlap with other manifestations of acute febrile syndrome. The isolation of local strains and further use in serological studies to the spirochetes will shed light weather tick-borne relapsing fever spirochetes do infect humans in Latin America.

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Symposium “Ecoepidemiology of Hantavirus Circulation in Latin America”

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Hantavirus pulmonary syndrome (HPS) is a zoonotic emerging infectious disease caused by Orthohantavirus associated to rodents in the Americas. The disease is characterized by a rapid onset of pulmonary edema followed by respiratory failure and cardiogenic shock. Due to the absence of vaccines against hantaviruses, the study of human immune responses and the ecology and parasitology of rodent hosts are essential. The distribution and emergence of diseases triggered by hantaviruses is determined by the dynamics of vectors and host populations, including complex processes such as the persistence of pathogens in the environment, geography, ecological interaction with other sympatric species, and climate. The knowledge regarding HPS has advanced greatly in the last two decades particularly in clinical and epidemiological findings, together with the identification of novel hantavirus lineages. More recently, cross sectional studies of rodent-to-rodents and rodent-to-humans ecological interactions have revealed key processes leading to species jumps and emergence of outbreaks. The perspective of one health is fundamental to take decisions about the prevention and control of the virus dissemination. In this symposium, Latin American researchers from Argentina, Brazil, Chile, and Panamá, cover these topics to contribute to the understanding of the disease and support the design of optimal disease control strategies.

Coordinators:
GÓMEZ VILLAFAÑE, Isabel E
TORRES PERES, Fernando

Speakers:
ARMIÉN, Blas
OLIVEIRA, Renata C
GÓMEZ VILLAFAÑE, Isabel E
VIAL, Cecilia

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Spatial distribution of *Oligoryzomys costaricensis*: Reservoir of *Choclo orthohantavirus*, cause of hantavirus disease in Panama, 1999-2022

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Background: The first cases of hantavirus pulmonary syndrome in Panama were diagnosed in early 2000 and *Oligoryzomys costaricensis* was identified as the reservoir for Choclo orthohantavirus. Surveillance of this disease has been carried out for 22 years and more than 400 cases have accumulated, mainly in the Center-West region of Panama. Objective: The objective of this work is to present the distribution of *Oligoryzomys costaricensis*, types of habitats and its implication in the development of hantavirus disease in Panama. Material and Methods: Between 2000-2020, captures were made with Sherman traps in a 10x10 arrangement in various habitats nationwide. Morphometric measurements were collected, and a blood and tissue sample were taken from everyone. We use immunoblot for the detection of IgG antibodies against hantavirus. Using ArcGIS 10.3 we generate thematic maps of altitude distribution, ecoregions, and land use. Results: We placed approximately 206,000 traps/nights at 156 locations and captured 6,937 individuals of 28 species. Of the specimens collected, 13.7% were O. costaricensis and of these 62% were males. The global prevalence of hantavirus detected was 15.7% (122/778, 95% CI = 13.1-18.2), being higher in males 19.4% (93/480) than in females 9.7%. (29/298). The highest prevalence of infection was detected in cultivated habitats (21.1%, 47/223), particularly below 100 meters above sea level. Discussion/conclusion: The results indicate that habitat fragmentation and the transformation of natural habitats into agricultural areas allow the increase and decrease of *O. costaricensis* populations, specifically in the highly disturbed lowlands of the Central-West Region of Panama. This region has extensive rice and pasture crops that support rodent populations that have annual and interannual variations, promoting the circulation and maintenance of the virus. On the other hand, CHOV circulates mainly in this region and corresponds to the area where most cases of hantavirus disease have occurred in Panama. The evidence generated from the One Health perspective is essential for decision makers to develop and implement cost-effective interventions for the prevention and control of future outbreaks of this pathogen in these regions.

Hantavirus in Brazil: an overview

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Hantaviruses are mainly rodent-borne viruses of great importance for public health, broadly distributed around the globe, responsible for the hantavirus pulmonary syndrome (HPS) in the Americas and hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia. Currently, 47 hantavirus species present in several vertebrate hosts with different ecological niches, such as rodents, shrews, moles, bats and more recently in reptiles and fish, are officially recognized. In Brazil, since the first identification of the disease in 1993, the number of hantaviruses identified in human and rodents' samples has increased, where more than 2200 cases of HPS was reported, with a mortality rate around 40%. Ten years before HPS was recognized, Seoul virus (SEOV) related to HFRS cases was identified in the specie *Rattus norvegicus* in Brazil, but no human case has been confirmed. Both HFRS and HPS are zoonosis under-diagnosed, especially in the Northern region of the country. The high prevalence of dengue, leptospirosis, influenza, and COVID-19 compromises the clinical and laboratory diagnosis of HPS and HFRS by health professionals due to the overlap of clinical signs, highlighting the importance of the differential diagnoses considering endemic diseases. The knowledge regarding HPS has advanced greatly in recent years in terms of clinical and epidemiological findings related to the disease and in terms of the identification of novel viruses and their host species through crosssectional studies and more recent studies on the ecology of reservoir populations. Brazil is a megadiverse country, where the diversity of mammals and especially rodents is considered one of the largest in the world. The family Cricetidae is the most diverse, with 117 species and 36 genera grouped in the subfamily Sigmodontinae, which includes all hantavirus hosts identified in the country. Notably, in most of Brazil, all the biomes have experienced rapid fragmentation, which may affect the ability of rodent species to spread throughout the landscape and may even affect species substitution. To date 8 hantavirus genotypes have been identified in rodent species belonging to the genus *Akodon*, *Calomys*, *Holochilus*, *Oligoryzomys*, *Oxymycterus* and *Necromys*. Although each hantavirus is predominantly associated with a specific rodent and a particular location, infections in other hosts by the same virus can occur by —spillover or interspecific transmission. In Brazil, there are also serological evidence of hantavirus in bats, and it is home to approximately 174 microchiropteran species, so the identification of novel hantaviruses in new reservoir bat species among the several Brazilian biomes remains a distinct possibility. In summary, the main factor correlated with human epidemics or small outbreaks of hantavirus appears to be an increase in rodent density, which promotes efficient propagation of the virus among the rodent population and a subsequent increase in the number of human cases. However, a simple increase in host rodent population density does not necessarily result in an increase in viral infection among the human population. The human activity behavior, the landscape use and the type of circulating hantavirus are also important factors for human infection confirming the complex set of interactions.

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Orthohantavirus variants and hosts in Argentina

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Hantavirus pulmonary syndrome (HPS) is an emerging infectious diseases caused by orthohantavirus associated to rodents of Cricetidae family, Sigmodontinae subfamily, in the Americans. It is characterized by a rapid onset of pulmonary edema followed by respiratory failure and cardiogenic shock. The virus is transmitted to humans via inhalation of aerosolized excreta of infected rodents. Virus transmission among rodents occurs mainly via saliva and blood and depends on rodent population density, structure, and behavior which, in turn, depend upon environmental conditions and food availability. The highest proportion of cases occurred in the Southern Cone of south America. Occasionally, spill-over events to non-traditional host can result in dead-end host, host-jumps or host-switch occurrences. In Argentina, the first cases of HPS in Argentina were recorded and characterized in 1995 in the southwest, but, since this first description different variants or genotypes of this virus have been described. Thirteen native species have been identified as orthohantavirus hosts, some of these are possible spillovers. Nowadays, the occurrence of cases is concentrated in four geographically isolated areas. In the north area (Salta and Jujuy provinces) there is the genotype Bermejo, present in the rodent *O. chacoensis* as the host, the genotype *Orán transmitted* in *O. occidentalis* and Laguna Negra in *Calomys callosus*; northeast area (Misiones) with the circulation of the genotype Juquitiba by the host *O. nigripes* and the identification of the Lechiguanas in humans, not associated with a rodent yet in the province; center area (Buenos Aires, Entre Ríos and Santa Fe provinces) where the three genotypes Lechiguanas, Buenos Aires and Plata are present, and the principal host is *O. flavescens*; and south area (Neuquén, Chubut and Río Negro provinces) with the genotype Sur in principally in *O. longicaudatus*. Additionally, in the center área two genotype non pathogenic are present: Maciel in Necromys obscurus and Pergamino in Akodon azarae. In recent years, hantavirus cases have been reported in three new provinces: Santa Cruz, Formosa and Corrientes. The distribution of diseases is determined by the distribution and dynamics of vectors and hosts populations, the persistence of pathogens in the environment and climate and weather characteristics. To date, there is no effective treatment or approved vaccine against HPS, and therefore, preventive measures are the only means to curtail human disease.

Host response to hantavirus infection, an OMIC approach

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New World hantaviruses are important human pathogens that can cause a severe zoonotic disease called hantavirus cardiopulmonary syndrome (HCPS). In particular, Andes virus infection produces HCPS where patients progress quickly to a severe condition with respiratory failure and cardiogenic shock that can be fatal in 30% of the cases. The role of the hosts immune responses in this progression towards HCPS remains elusive. We characterized this immune response during the acute phase of the disease, and did an unbiased study of a subset of 12 patients hospitalized with severe HCPS. This unbiased study was a transcriptome analysis of PBMCs of these patients, which were combined with clinical laboratory data to gain a better insight into factors associated with a severe clinical course. Patients were further classified in two levels of severity, a first group that required mechanical ventilation and vasoactive drugs (VM+VD) and a second group that also needed ECMO or died (ECMO/Fatal). Their transcriptional profile was compared during acute (early and late) and convalescent phases. Our results showed that overexpression of the interferon response is correlated with a worse (ECMO/Fatal) outcome and an increased viral load and proinflammatory cytokines in the early-acute-phase. This study provides insights into the differences in innate immune activation between severe patients that associates with different clinical outcomes, using a non-biased approximation.

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Symposium "Dangerous mimicry: Leptospirosis and vector-borne febrile diseases in Latin America"

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The tropical and subtropical climate of Latin American countries favors the co-circulation of several infectious diseases such as Malaria, Dengue, Chikungunya, Zika, leptospirosis and others. All these share non-specific febrile symptoms and signs that make their diagnosis and differentiation difficult. The emphasis that has been given to certain vector-borne diseases has led to the under-diagnosis of leptospirosis and reduced awareness of the impact of this disease in the region. During this symposium, researchers from four South American countries will share the results of their research on leptospirosis performed under the One Health approach. We aim to characterize the ecoepidemiology of leptospirosis by highlighting the heterogeneity of its reservoirs, hosts, and environments in which it occurs. The topics discussed during the symposium will contrast the problematic of leptospirosis with other endemic febrile vector-borne diseases in the region. The impact of the COVID-19 pandemic on leptospirosis surveillance in the four countries will be discussed.

Coordinators:
BARRAGÁN, Verónica
PREVITALI, Andrea

Speakers:
BARRAGÁN, Verónica
AGUDELO-FLOREZ, Piedad
PREVITALI, M Andrea
COSTA, Federico

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The unexpected epidemiology of rural leptospirosis

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The diversity of animal reservoirs, the behavior of human communities and their relationship with the environment are determining factors in the epidemiology of leptospirosis. Leptospirosis in urban settings has been widely studied, and rats have been defined as the most important and abundant reservoirs of pathogenic leptospira species. In contrast, rural leptospirosis has been scarcely studied, probably due to the complexity that results from the diversity of animal reservoirs and pathogen. In the last decade, we have studied rural leptospirosis in tropical areas of Ecuador-South America. In low-income rural communities on the coast of Ecuador, we have found entire families highly exposed to a high diversity of leptospira species. Serum and urine samples were collected from household inhabitants and from animals living in the peridomestic environment. Microagglutination Testing (MAT) results showed that 100 % of pigs, dogs, and cows, and 47% of humans have been exposed to leptospirosis. Moreover, 23.7% (95% CI: 0.22-0.49) of these animals (n=59) excrete the spiroquete in their urine. The scenario is similar in the Amazon basin, where we analyzed domestic dogs of the Kichwua and Waorani communities as sentinel animals for leptospirosis risk to humans. These dogs feed on the remains of animals hunted by the community and as a consequence are exposed to pathogenic leptospira species circulating in wildlife. We have found that 85% (95%CI: 0.73 -0.97) of these dogs (n=34) excrete the bacteria in their urine, consequently, pose a high risk of infection to the community. Unfortunately, leptospirosis remains a neglected and misdiagnosed disease in the Americas and is often confused with other febrile illnesses, making it difficult to know the true prevalence of the disease in the human population. This situation is probably more severe in rural areas, where the close relationship between animals and humans and the high percentage of animals excreting the pathogen increase the risk of infection. The results of this study highlight the importance of understanding the diverse epidemiology of leptospirosis in rural areas. It also highlights the importance of monitoring the excretion of the pathogen in urine of domestic and wild animals of rural areas, as well as to educate the population on proper animal handling and proper use of footwear.

Leptospirosis in northwestern Colombia seen from the human-animal interface: new species and new actors

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Leptospirosis is a zoonotic bacterial disease that affects both humans and animals, caused by various species of the genus *Leptospira*. The disease is known to be endemic in some tropical and sub-tropical Latin-American countries; Leptospirosis constitutes one of the major causes of febrile illness in Colombia, and therefore, there is an urgent need to implement surveillance that can be used to recognize leptospirosis in regions such as the Urabá gulf region in Colombian northwestern that has several conditions that are favorable for the transmission of the disease, including little urbanization, a large population of potential reservoirs, and frequent flooding conditions favoring interaction at the human-animal interface. In our research in the region, we detected human *Leptospira* spp. antibodies by microagglutination with a seroprevalence was 12.5% (95%CI: 10.01-15.5). No differences were observed according to race, gender, occupation, age, living conditions, or time of residence in the area. *L.interrogans* and *L. santarosai* were the most prevalent species. Also, when we study of social and environmental risk factors associated with leptospirosis of inpatient and outpatient management in the Urabá we described that the presence of wildlife in homes could also be related to cases of inpatient management. This finding represented 4.22 (1.13 to 15.72) times the risk compared with outpatient cases reported as not having this type of animals inside their home (p=0.032). We established the etiology and clinical signs of acute febrile non-malaria syndromes and explore some of the likely risk factors in patients which exhibit these symptoms and come from that region. In these works, we found that the frequency of infection for leptospirosis was 37.3%; We stablished different diagnosis based in PCR assay using 16S Ribosomal gene and we proposed a new target, *rpoC* gene. It was a valuable tool for the detection and identification of *Leptospira* species directly to bats as reservoir hosts of *Leptospira* and to evaluate the genetic diversity of circulating species. We captured 206 bats and twenty bats tested positive for *Leptospira* spp. infection (20/206-9.70%) and the species of infected bats and the infecting *Leptospira* species identified were *L. borgpetersenii*, *L. alexanderi*, *L. noguchii*, *L. interrogans* and *L. kirschneri*. Our results confirm previous records that underline the importance of *Leptospira* spp as causal agents of febrile syndrome in this region of Colombia. To recognize the *Leptospira* serovars or genomospecies associated with bats in endemic tropical areas should be prioritized for understanding the epidemiology, ecology, and evolution of *Leptospira* in this host-pathogen association. These studies suggest a basis for designing and implementing effective interventions and optimize prevention and control strategies.

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Study of the co-occurrence of leptospirosis and dengue in neighborhoods of Santa Fe, Argentina: a participatory action research work

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Leptospirosis is a zoonotic disease of global distribution caused by bacteria of the genus Leptospira, acquired by direct or indirect contact with urine of infected mammalian hosts. On the other hand, dengue fever is a mosquito-borne viral disease caused by the Flavivirus DENV. Both leptospirosis and dengue fever have similar symptoms at the onset of the disease. The city of Santa Fe, Argentina is endemic for leptospirosis and it also has experienced dengue epidemics in recent years. Late summer and fall are the seasons when most cases occur. We used a One Health approach to investigate leptospirosis and dengue in three marginal neighborhoods of the city of Santa Fe by considering aspects of animal, human and environmental health. Our goal was to compare how people perceive their risk of contracting these diseases with their exposure to these pathogens. We used a participatory action research program involving several sectors and disciplines with the purpose of considering multiple dimensions of the problematic associated with the co-occurrence of leptospirosis and dengue in the territory. Our approach involved the analysis of biological samples, surveys and observations made in the field. Additionally, we used interviews, workshops, and collective mappings to inquire about public risk perception. The articulation of the two approaches enabled obtaining results from samples collected from sites relevant for the community, integrating popular knowledge on the biology of the synanthropic mammals with those obtained from traditional methods, and to consider common human -wildlife interactions when evaluating potential transmission risk. We collected a total of 191 blood samples and the estimated seroprevalence for leptospira exposure was 8.9% and for dengue virus was 16.2%. Additionally, 24% of the water samples collected were positive for pathogenic leptospires based on our qPCR results. Our surveys showed that the perception of the risk differed among the three neighborhood studies. However, in all three the majority of people noted that there are more prevention campaigns for dengue than leptospirosis. We present some additional insights gained on the exposure to these zoonotic pathogens and discuss some of the challenges faced when working collectively with citizens in promoting actions to reduce their risk.

Epidemiology of urban leptospirosis

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In this project we performed a systematic interdisciplinary evaluation of the reservoir host, pathogen, environment and human-related factors needed to understand the timing, location and intensity of leptospirosis epidemics. This project characterized the ecological factors driving leptospire dynamics and shedding in rat populations, the survival of leptospires on environmental reservoirs, and influence on human infection to develop and test community base interventions aimed to prevent leptospirosis transmission. (1) We have stablished the demography, transmission dynamics and shedding of leptopsires in the *Rattus norvegicus* reservoir. Our results show a notable lack of seasonal variation in reservoir dynamics, though rat activity varies at fine geographical scales. Rats have high leptospire carriage rates (>80%), and high rates of leptospire shedding. Novel, more detailed analysis suggested that vertical and environmental transmission occur in the wild at epidemiological significant rates. These studies have now been combined to construct a mechanistic mathematical model to account for variations in leptospire shedding by rats. (2) We characterized the leptospires dynamics in the environment, a key component of rodent and human transmission. Once shed in the urine of infected rats, the environment serves as an important reservoir for leptospires. Depending on factors such as pH, salinity, temperature and humidity, leptospires can survive for 1-2 weeks. We developed and standardized protocols to quantify pathogenic leptospires, which we detected in in soil and water samples from Pau da Lima. We found significantly greater proportions of qPCR positive samples and higher leptospiral concentrations when samples were from sewage and collected during periods of increased rainfall. (3) We established a link between the spatio-temporal variations in the rat activity and in the pool of leptospires shed by rodents and human risk for Leptospira infection. By densely sampling track plates (an innovative method developed in this project to quantify rodent activity), we were able to create a surface of rat activity with high spatial resolution which was associated to the risk of Leptospira infection. Rat infestation had a linear association with the risk of Leptospira infection. We also observed that leptospire shedding estimates were correlated to with the incidence of Leptospira infection of residents during the periods of low rainfall. All this data together suggests that the relationship between rodents and human risk is modified by other temporal components as rainfall. (5) Most recently, we have performed applications of optimal control theory, seeking to determine the consequences of various management practices (controlling either rats or the environment) such that optimal interventions (including both costs and intervention time-courses) can be identified. The aim of our model was to reduce total rat population size, the quantity of shed leptospires and hence the risk to humans, in the most cost-effective way given the cost of control measures and of each infected rat. As an initial proof-of-concept, we investigated five different control scenarios, combining rodenticide and habitat management applied alone, simultaneously or sequentially. The model works in that it is theoretically operational suggesting that applying rodenticide alone as the most cost-effective approach. (6) We tested in the field the effectiveness of an intervention to control rodents performed by the city government. The intervention shown a decrease on rat activity after the intervention as well as evidence of severe genetic bottlenecks, with effective population size dropping after eradication. We are now doing follow-up statistical analyses to better understand the impact of the intervention on human transmission.

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Symposium "Dead the dog, is the Rabies gone?"

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In Latin America, the decrease in rabies transmitted by dogs through national vaccination campaigns and responsible pet ownership measures has highlighted the importance of other wild mammals in maintaining this disease. The biodiversity of possible wild reservoirs of the rabies virus has increased in the last 10 years, including taxa that originally were not considered within the phenomenon (i.e. primates in Brazil), due to the development and advances of molecular diagnostic techniques and the expansion of surveillance systems. However, the role of these species as an emerging reservoir of the disease is not clear since there is a lack of evidence and knowledge regarding whether they are responsible for its maintenance (reservoirs) or are only accidental vectors. In this Symposium, new reservoirs identified in Argentina, Brazil and Mexico will be presented, and we will discuss the challenges that they could constitute for their prevention and control.

Coordinators:	CISTERNA, Daniel ARÉCHIGA-CEBALLOS, Nidia
Speakers:	BENAVIDES, Julio PUEBLA, Paola K APPOLINARIO, Camila M CARABALLO, Diego A

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Emerging challenges for the control of rabies in wildlife of Latin America

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Rabies transmitted by wild animals has become the main source of human deaths associated with rabies in Latin America. Most human and livestock deaths are caused by rabies transmitted by the common vampire bat *Desmodus rotundus.* However, new wild reservoirs of rabies are also emerging, including the common marmoset *Callithrix jacchus* and the crab-eating fox *Cerdocyon thous*. Moreover, cases of secondary transmission of rabies involving wild and domestic reservoirs have been recently reported (e.g. bat to cat to human). In this talk, I summarize several emerging challenges associated with the prevention and control of rabies transmitted by wildlife in Latin America including i) evidence of rabies spatial expansions among several wild reservoirs, ii) poor understanding of the rabies transmission dynamics within some wild species, and iii) poor knowledge among health professionals prescribing post-exposure prophylaxis when assessing a patient bitten by a wild animal. I discuss avenues for future research under a One Health approach that could help optimizing the prevention and control of human and livestock cases of rabies transmitted by wild animals.

Are coatis (*Nasua narica)* emergent reservoirs of rabies virus in Mexico?

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In 2019, Mexico was recognized by WHO /PAHO as a human rabies -free country transmitted by dogs. Nowadays, the challenge is to control rabies virus (RV) transmitted by wildlife to humans and domestic animals. The white-nosed coati (*Nasua narica*) is a carnivore that belongs to the Procyonidae family. Rabies cases in this species have been poorly investigated in Mexico. Since 2007, there have been seven reported cases of rabies occurred in coatis in the states of Yucatan (YUC) and Quintana Roo (ROO). After an outbreak in ROO in which a new rabies virus variant (RVV) called "V9-Coati" related to the insectivorous bat *Tadarida brasiliensis* was described. The aim of this study was to characterize antigenically and genetically the registered cases of rabies in coatis in YUC and ROO to determine if these cases correspond to a single or multiple RVV. A total of seven samples of coatis (2007-2020) from YUC (n=4) and ROO (n=3) were antigenically characterized with the Monoclonal Antibodies panels (CDC). The genomic characterization of the coati RV samples was performed by amplifying a fragment of nucleoprotein gene using RT-PCR and sequenced by the Sanger method. Phylogenetic reconstruction was based on a dataset of RV partial nucleoprotein sequences belonging to RVV of the Americas. The Bayesian analysis was carried out using the Beast 10 software by the evolutionary model Kimura 2P+G. The samples from YUC were RVV atypical and were phylogenetically clustered in the lineage that has been previously designated as "Yucatan Sylvatic" associated with the terrestrial RV cycle. This RVV atypical have been responsible for the last rabies cases in domestic animals in YUC, in which skunks seem to be the active transmitters. The 3 samples from ROO were RVV9 and were phylogenetic clustered in the lineage "V9-coati" pertaining to aerial cycle. In YUC and ROO, two independent RVV were detected in coatis. There is no evidence of overlapping between them; each one seems to have a restricted distribution. Due to the presence of an outbreak in ROO, one year before being described in 2008, RVV9 of coati we hypothesized this is a host switching from insectivorous bats to coatis. Nevertheless, the origin of this virus has not yet been determined since the natural distribution of *T. brasiliensis* in Mexico does not include these states, therefore we consider bats in the Molossidae family might be the most probable origin. So far, coatis have not been considered important RV reservoirs; our results suggest that a new lineage has been established in coatis of ROO, therefore surveillance in coatis should be relevant in the control of rabies in Mexico.

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The new face of rabies in Brazil

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In Brazil, urban rabies was considered an important public health problem in the second half of the 19 century, and dogs were considered the most important transmitter of the disease to humans. In 1973 Brazilian government established the National Program for Rabies Prophylaxis with annual mass vaccination campaigns for dogs and cats as one of the main pillars of the program; in 1977 the program was present in all national territories with a reduction of human and dog/cat rabies cases. It is important to notice that only after the program, notification of human and animal cases of rabies become compulsory. Incidence in dogs dropped from 1,900 cases in 1999 to 11 cases in 2020. Between 1980 and 2021 1,487 human rabies cases were registered in Brazil, but there was a shift in epidemiology regarding the variant involved in those cases. Up to the year 2000, most cases were transmitted by a dog and caused by a dog variant (AgV2), but this epidemiological profile changed after that. A retrospective descriptive study of the series of cases of human rabies recorded from 2000 to 2017 resulted in 188 human cases; the majority of cases (85.6%) occurred in the period 2000-2008, 45.7% involving dogs and 43.6% hematophagous bats. Of these, 85.1% occurred from five outbreaks in riverside populations in the north of the country between 2004 and 2005. From 2009 to 2017, there were 27 cases (14.4%); of these, 40.7% involved dog aggression, 29.6% bats, 14.8% monkeys and 11.1% cats, predominating bat Agv3 when virus isolation and characterization were possible. In 2018, 11 deaths were notified, being 10 from an outbreak in Amazon region, as occurred in the previous year; all 11 deaths had the hematophagous bat identified as the aggressor. In 2019, one human death was registered involving a cat as the aggressor, but viral isolation and characterization identified AgV3 as the involved variant, confirming the importance of secondary transmission in the epidemiological scenario of rabies in Brazil. In 2020, two cases were registered, one related to bat aggression and the second one involving a crab-eating fox and its specific non-classified variant. In 2021, one case involving again a crab-eating fox in the state of Maranhão, where interestingly, dog rabies is still considered endemic. In the current year, in May, three cases in an indigenous community were notified, involving direct aggression of hematophagous bat. Considering the continuous increase of rabies cases transmitted by the hematophagous bat, the growing number of bat populations in urban areas, the importance of secondary transmission of rabies by cats, and that mass vaccination campaigns against rabies for dogs and cats are no longer part of the National Program for Rabies Prophylaxis, adjustments in the epidemiological surveillance and awareness of general population and veterinarians are important in this new face of rabies in Brazil.

Rabies virus variants maintained by dogs and foxes in South America

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The rabies virus (RABV) is characterized by a history dominated by host shifts within and among bats and carnivores. One of the main outcomes of long-term RABV maintenance in dogs was the establishment of variants in a wide variety of mesocarnivores. In this study, we present the most comprehensive phylogenetic and phylogeographic analysis, contributing to a better understanding of the origins, diversification, and the role of different host species in the evolution and diffusion of a dog-related variant endemic of South America. A total of 237 complete Nucleoprotein gene sequences were studied, corresponding to wild and domestic species, performing selection analyses, ancestral states reconstructions, and recombination analyses. This variant originated in Brazil and disseminated through Argentina and Paraguay, where a previously unknown lineage was found. A single host shift was identified in the phylogeny, from dog to the crab-eating fox (*Cerdocyon thous*) in the Northeast of Brazil. Although this process occurred in a background of purifying selection, there is evidence of adaptive evolution -or selection of sub-consensus sequences- in internal branches after the host shift. The interaction of domestic and wild cycles persisted after host switching, as revealed by spillover and putative recombination events.

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Symposium "Control of Cystic Echinococcosis: Prospects"

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Echinococcosis is an important zoonotic parasitic disease. It is caused by different species of the taenia *Echinococcus* spp. Neotropical Cystic Echinococcosis (NCE) is due to *Echinococcus vogeli*, while Alveolar Echinococcosis (AE) is related to *Echinococcus multilocularis*. In South America, NCE has been reported but there has been no intervention to study and control this parasite in the region. On the other hand, AE has not been reported in South America yet. Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is a chronic and debilitating zoonotic larval cestode infection in humans, which is principally transmitted between dogs and domestic livestock, particularly sheep. Human CE occurs in almost all pastoral communities and rangeland areas of the underdeveloped and developed world. Control programs against CE have been implemented in several endemic countries to reduce or eliminate the disease. New Zealand and Tasmania are examples of some of the first programmes to be undertaken (in insular territories) and which were very successful in the elimination of CE. The advent and proven effectiveness of praziquantel, plus the experience of insular models, produced high expectations for rapid advances in a second generation of control programmes undertaken in continental areas (Argentina, Uruguay, and Chile). Nevertheless, only moderate gains in CE control have been made and the impact on prevalence among humans has been slight. A major impediment to the adoption of procedures that were successful in New Zealand and Tasmania has been the requirement to administer praziquantel to dogs in rural areas 8 times per year over numerous years. In addition, there have been clear technological improvements made in the diagnosis of canine echinococcosis for surveillance, the genetic characterization of parasite strains and in vaccination against CE infection in livestock. In order to establish new paradigms and appropriate combinations of control strategies we have carried out a review and discussion of the available control tools and control models. Control strategies must be suitable and sustainable to benefit the Echinococcosis-endemic areas primarily, which at the same time are the poorest regions of the world.

Coordinators:
LARRIEU, Edmundo
GAVIDIA CHUCÁN, César

Speakers:
VIZCAYCHIPI, Katherina
JERCIC, María I
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VIGILATO Marco AN

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Status of neotropical echinococcosis, emerging zoonotic disease

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Neotropical echinococcosis (NE), polycystic, caused by *Echinococcus vogeli* and unicystic, caused by *Echinococcus oligarthra*, produce an emerging zoonosis in human populations in 15 tropical countries of Central and South America. These species differ from the others of the genus *Echinocococcus* due to their epidemiology, life cycle, cist morphology and rostellar hooks, clinical manifestations and course of the disease in humans. In relation to epidemiology, the human groups most exposed to the risk of acquiring this echinococcosis live in rural areas of tropical forests with the presence of natural transmission hosts and knowledge of wild animal hunting (paca, agouti). Is important to consider that, the people who migrate to the urban centers of jungle areas (which are actually small towns), continue with the cultural habits of risk similar to those previously maintained in the jungle. The increase in anthropic activities for economic purposes in the wild habitat enlarge the interface between wildlife and humans. Being able to increase the number of infected with zoonotic diseases, as well as influence and modify the natural cycles of transmission of the NE. The popularity of the domestic cat will undoubtedly influence the dissemination of *E. oligarthra*. Polycystic neotropical echinococcosis is one of the most important lethal zoonotic of Central and South America, reaches the death of the patient in the third part of the cases and this high mortality rate is due to the aggressiveness of the parasite and the deficiency of clinical recognition of human infection. The aforementioned reinforces the need for integral approaches, which allow the optimization of information paths and awareness of the real dimension of EN, increasing the necessary management strategies for prevention, diagnosis, and control.

Advances in the diagnosis of Cystic Echinococcosis in the definitive host

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The diagnosis of Cystic Echinococcosis in the definitive host is an essential component of surveillance data that indicates the burdens of the disease and allows progress and success evaluation of control programs, being an important instrument to support measure controls of this parasite disease. In risk areas, it is done in two occasions, first as a base line, and then to evaluate the intervention that has been done. Traditionally, infection in dogs has been determined by identifying worms in intestinal washes postmortem or after arecoline administration, then different immunological methods were introduced e.g., enzyme-linked immunosorbent assay (ELISA) for detection of parasite antigens in feces (copro-ELISA), or molecular techniques such as Polymerase Chain Reaction (copro-PCR). Coproantigen tests are genus specific with a specificity of approximately 97%. However, sensitivity is relatively limited, resulting in an overall average test sensitivity of only 60% for natural canine *Echinococcus granulosus* infection. These tests have facilitated large scale screening of definitive hosts, but the sensitivity and species-specific detections must be improved. Molecular methods became a new alternative to improve the diagnosis to canine Echinococcosis. Several methods have been published, such as conventional PCR, Real Time PCR; and recently Loop-mediated isothermal amplification (LAMP). The main obstacle for molecular techniques success is the obtention of a good sample and DNA extraction due to the difficulty of breaking the eggs. New surveillance control programs emphasize on risk zones identification rather than the identification of infected dogs. Extraction methods should be able to avoid PCR-inhibitors present in environmental samples. Thus, flotation techniques appear as an option to clean samples and improve the sensitivity prior samples analysis. There are several alternatives to select specific DNA primers for detection of *E. granulosus*, which has been published in the last years. However, the most important problem is the cost of implementing molecular techniques. It requires a specific laboratory with separate rooms and specifically designed instruments, in addition to expert professionals. A new LAMP technique offers a simpler and lower cost alternative that doesn't require a lot of equipment to develop. In conclusion, the heterogeneity of canine Echinococcosis diagnosis across the time and the sources of important variability merit further attention and research to develop better surveillance standards that operationalize the Plans of Action to control this parasite neglected disease.

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The adaptability of control programs for Cystic Echinococcosis according to different geographic areas and population dynamics

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The hydatidosis program of the Province of Río Negro, Argentina, has been in force for more than 40 years. Although, since its inception, the prevalences in the different hosts have decreased notably, it has not yet been proposed to implement an eradication program in Río Negro. There is, however, the concept of keeping the prevalence contained or controlled, but in the face of changes in some variables, such as a decrease in the presence of health effectors in the field, migration of the rural population to urban centers, climate change, pandemic with isolation prolonged preventive, the appearance of human cases occurs again (spring effect). Taking into account the demographic variables, the current diagnostic techniques and health policies of the Provincial and National Ministries of Health, it is that the field technical references are modifying the approach strategies in terms of prevention and promotion that are being applied and evaluated every year.

Cystic echinococcosis in South America: the commitment of the countries in developing a Regional Program

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For many years, the Pan American Health Organization (PAHO) has recognized Cystic Echinococcosis/Hydatidosis as an important health problem in South America. In 1999, at the XIX International Congress of the International Association of Hydatidology, the Director of PAHO convened a working group to review and analyze experiences on perspectives and possibilities for the control and eradication of Cystic Echinococcosis/Hydatidosis. As a result, at the Inter-American Ministerial Meeting on Health and Agriculture (RIMSA XII) in 2001, recommendations were made for countries to continue strengthening the Veterinary Public Health approach. Within this framework, the First Meeting of Heads of Cystic Echinococcosis/Hydatidosis Programmes, convened by PAHO and held at the Pan American Center for Foot-and-Mouth Disease and Veterinary Public Health (PANAFTOSA/VPH-PAHO/WHO) in 2001, produced a document entitled "Plan of Action and Basic Regional Strategies for the Elimination of Human Hydatidosis in South America". The Constitutive Meeting for the Southern Cone Subregional Project for the Control and Surveillance of Hydatidosis was held in 2004. In 2013, the Subregional Project became the South American Initiative for the Control and Surveillance of Cystic Echinococcosis/Hydatidosis, incorporating new countries and neotropical echinococcosis (NE). Twelve meetings of the Initiative have been held, bringing together representatives of the countries and specialists in each of the topics. Since then, expert members of the Initiative highlighted the need for a regional program for the control and elimination of Cystic Echinococcosis in the Southern Cone. In addition, PAHO included the disease in the "Action Plan for the Elimination of Neglected Infectious Diseases and Post-Elimination Actions 2016-2022", with a focus on surveillance, diagnosis, management and prevention of cases. In this regard, a group of official focal points has recently been formed which, under the coordination of PANAFTOSA/VPH-PAHO/WHO and technically supported by the Initiative, developed the Regional Program for the Elimination of Cystic Echinococcosis/Hydatidosis 2020-2029 and the corresponding Regional Action Plan. The objective is to align the strategic lines of action and monitoring progress in the fight against the disease in six countries: Argentina, Brazil, Chile, Paraguay, Peru and Uruguay. The goals, expected results and performance indicators of the Program established in the Action Plan are based on four strategic intervention axes: (1) strengthening surveillance and diagnosis in humans and animals; (2) attention to human cases; (3) interventions in animal hosts; (4) health education. These outcomes were defined according to the most common intervention points for breaking the transmission cycle of the disease and consequently reducing its incidence and prevalence. The goals for each axis of intervention, the expected results and the year of execution were defined and adapted according to the needs and reality of each country. The countries committed themselves to strengthen their National Program, and align their strategies with the goals and actions agreed in the Regional Action Plan. The implementation of the Regional Program aims to achieve progress in the control and elimination of Cystic Echinococcosis/Hydatidosis as a public health problem by the dates foreseen in the Regional Action Plan.
More information: https://www.paho.org/en/topics/hydatidosis-echinococcosis

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Symposium "Phytosanitary surveillance of plant pest vectors"

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This Symposium presents real examples of phytosanitary threats caused by two pests, huanglongbing (HLB) of citrus fruits and *Xyllela fastidiosa* in olive trees, and their respective vectors in Argentina, which are currently being officially managed by the National or Provincial State. An update of the preventive tools that are carried out for early detection of exotic pests is also included. Through these four examples this Symposium aims to provide a brief synthesis of the strategies that are being carried out by the State for the protection of plant health in Argentina.

Coordinator:	CORTESE, Pablo
Speakers:	OUTI, Yanina S DEFEA, Bárbara S & ROCA, Mónica EM RAMIREZ, Wilda & AUGIER, Lucrecia CORTESE, Pablo

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Plant protection: vector survaillance and vector analysis for the early detection of diseases

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The presence of vectors plays a fundamental role in determining the phytosanitary risk of phytopathogens in Argentina and is decisive for decision-making regarding the incorporation of a disease in the prioritization of pests for phytosanitary surveillance whose purpose is to define which pests/diseases and For what purpose will they be actively monitored by SENASA. Within the framework of this prioritization, in recent years diseases have not been incorporated because the absence of vectors limits their dispersion and development in the territory, as in the case of *Grapevine flavescence* dorée phytoplasma, the causal agent of one of the most important yellowing diseases of the vines in Europe, since its vector *Scaphoideus titanus* is absent in Argentina and South America. In this case, both organisms are currently included on the list of quarantine pests absent in Argentina and are regulated in the importation of plant products to prevent their entry into the territory. When disease vectors are present, the risk of their establishment and spread is greater. Some of the diseases and their vectors that were prioritized and in which the presence of vectors played a special role are: HLB, *Xylela fatidiosa* and Candidatus *Phytoplasma phyri*. Regarding the citrus HLB, the actions began in 2009, at the time of prioritizing the disease, the risk of entry was very high given the proximity of the disease to the border with Brazil, and its vector *Diaphorina citri* was present in the northeastern region of Argentina. Surveillance was based on a network of yellow chromatic traps, and as an additional advantage, taking samples of the vector allowed it to be analyzed for the presence of the causal agent of HLB Ca. *Liberibater asiaticus*. As for *Xylela fatidiosa*, this bacterium is an exclusive inhabitant of the xylem and is transmitted by xylem-feeding insects. The different sub. species of this bacterium and have a wide range of hosts and a large number of vectors of the Order Hemiptera, families Cicadellidae and Cercopidae. Of this bacterium in Argentina, only the subspecies has been detected pauca. Surveillance was based on a sampling of plant material, extracting 1953 samples analyzed in crops. Due to the large number of vectors present, VID and Stone fruits crops are currently under surveillance in order to detect other subspecies of the bacterium at an early stage». Candidatus *Phytoplasma phyri*, causal agent of Pear decline, was detected in Argentina in 2019 (FD Fernandez 2019), has *Cacopsylla pyri, C. pyricola and C. pyrisuga* as vectors. Currently there is a surveillance system of 64 trapping sites with more than 200 revisions of traps. Knowing the presence and distribution of the vectors will allow knowing the areas of potential distribution of the disease and having information for risk analysis and decision making.

Xylella fastidiosa in olives crops from Argentina: epidemiology and potencial vectors

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Rapid Decay symptoms in olive trees caused by *Xylella fastidiosa* (*Xf*) infection have emerged as a potential threat to one of the most important olive production area in Argentina. Since 2013, a marked decline of the traditional cultivar was observed being affected both, old and young orchards. Monitoring surveys and diagnostic tests has determined the prevalence of *X. fastidiosa* in areas from La Rioja, Catamarca, Córdoba and Buenos Aires provinces. In La Rioja province, the local cultivar Arauco is the most affected by the bacterium that was also detected in others cvs. Manzanilla, Arbequina Picual, Frantoio, Barnea and Aloreña. The subspecies *pauca* (ST69 and ST78) were detected in olive plants and have only been found in Argentina. The ST69 type sequence was also detected in orange trees from the northeastern region and the ST78 in almonds in Catamarca. The main vectors of X. fastidiosa are xylem-sap feeders belonging to the families Aphrophoridae, Cercopidae (spittlebugs), and Cicadellidae Cicadellinae (sharpshooters) (Hemiptera: Auchenorrhyncha). In order to identify the potential vectors to olive plants a survey was conducted in orchards positive to the bacterium located in La Rioja province (Aimogasta and Chilecito). Since 2016, xylem-feeding hoppers are collected from olives trees with sticky yellow traps and from the surrounding vegetation with entomological sweep nets. The collected specimens were identified by the specialists of the Auchenorrhyncha laboratory of MLP (Entomology Department). Until know, the subfamily Cicadellinae (Cicadellidae) was the most represented and species of the genera *Macugonalia* Young, *Scopogonalia* Young and *Molomea* China were the most abundant in the canopy and associated vegetation. The sharpshooter *Bucephalogonia xanthophis* (Berg), a vector of *X. fastidiosa* in Brazil along with other species tested positive for the bacterium in northeastern Argentina were also recorded. Specimens of *Notozulia entreriana* (Berg) (Cercopidae), a confirmed vector of the bacterium in Brazil, were collected in the spontaneous vegetation associated with the crop. To proceed, the identification of the key vectors along with biological and phenological information are necessary to determinate best control options and evaluate risks at local and regional scale to avoid the spread of the bacterium to those areas where it has not yet been detected.

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Citrus fruits endangered by HLB

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NATIONAL HLB PREVENTION PROGRAM (PNPHLB for its acronym in Spanish). Citrus growers, who have been engaged in this activity for generations, are threatened by HLB, the most destructive citrus disease for which there is no cure. The advance of the disease in some citrus productive areas of Argentina is very worrying, and the establishment of such disease without any type of control measures can cause a 40% reduction in productive capacity in 5 years. To protect the national citrus production, SENASA (National Agrifood Health and Quality Service) coordinates and executes the National HLB Prevention Program (PNPHLB for its acronym in Spanish), created by Law 26,888, since 2010. To this end, a surveillance and monitoring system has been implemented for the early detection of HLB and its vector (*Diaphorina citri),* which to date has more than 472,349 sites monitored and almost 60,855 samples taken, of which 1,314 plant samples and 67 Insect samples were positive for the presence of *Candidatus Liberibacter asiaticus.* In addition to the visual inspection of HLB and its vector, the surveillance system has a network of 317 trapping sites for monitoring the insect vector in areas where it is absent, along with a complementary network from the Obispo Colombres Agroindustrial Experimental Station (EEAOC) and the private sector, to join the official network of SENASA, and which has approximately 3,600 monitoring sites in commercial citrus orchards in Tucumán, Salta and Catamarca, and some 87 sites in the urban trees of Tucumán, in order to detect the incursion of the pest and to be able to implement measures for its containment and/or eradication. *Diaphorina citri*, Kuwayama (Hemiptera: Liviidae) is the main vector of the bacterium causing HLB. Direct damage results from sap extraction, while the indirect and primary damage is done by transmitting the pathogen that circulates through the phloem of the plants. Detection of the insect is fundamental to determinate its presence and/or take control decisions. There are different techniques for its monitoring: visual inspection and sticky color traps. The former allows searching for the vector in its three states: eggs, nymphs and adults, while the latter captures the adult only. The analysis of the presence of this bacterium is carried out by the official laboratory network comprising INTA Concordia, Bella Vista, Montecarlo and Yuto, the EEAOC and the central SENASA laboratory. This monitoring and the coordinated actions carried out by public and private Institutions that are part of the national citrus chain are key to the implementation of public policies that SENASA establishes to protect the national citrus production. What is more, they are the basis to prevent the spread of the disease, avoiding the impact on regional economies, allowing to face the appearance of outbreaks of the disease quickly and effectively. These efforts must be strengthened with the commitment of the producers, through the adoption of preventive measures such as lot monitoring, vector insect management, and the acquisition of certified plants.

Phytosanitary Intelligence applied to the potential distribution of plant pest vectors

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Defining the risks of pests and their vectors with sufficient anticipation and precision is a critical aspect for the National Plant Protection Organizations (ONPF), which, among other functions, implement surveillance actions to prevent pest outbreaks. Phytosanitary intelligence is a relatively new concept within the International Phytosanitary Convention (IPPC). This term refers to knowledge management to assist in making decisions required to manage phytosanitary risk. To carry it out, a set of tools for its implementation have been developed so far, within the framework of official management:

- Prediction models: that work on a mathematical-statistical basis that relates the occurrence event with the values of anthropogenic and environmental variables that influence the distribution of the species, in a geographic information environment (GIS).
- Bio statistical indicators: built by algorithms that relate different epidemiological, biological and ecological aspects of the pest.
- Interactive platform that allows the exploration of a geospatial database for the estimation of areas with potential risk of establishment of exotic pests and their vectors, considering historical data and different climate change scenarios. This tool is available at (*http://sigafit.senasa.gob.ar/3.0/)* and predicts scenarios for the Argentine Republic and South America from 20 to 60 years ahead.

Within the framework of the official management of phytosanitary threats, phytosanitary intelligence is making it possible to attend to and manage the risk of pests of national and regional importance. Within this framework, work has been done to define the vector of the bacterium that causes HLB in citrus fruits *(Diaphorina citri*) and the vector of several solanaceous diseases (*Bactericera cockerelli*), among others. The aforementioned tools have been developed in the last 12 years by the multidisciplinary team of the Phytosanitary Strategic Information Directorate of the National Service for Agrifood Health and Quality, (SENASA) which has worked on the implementation of "phytosanitary intelligence", with the aim of strengthening the protection of the national phytosanitary patrimony.

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Symposium "Arthropod parasites and arthropod vector-borne diseases with veterinary importance"

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Arthropod parasites and arthropod vector-borne diseases constitute a major constraint to animal health and production. They are relevant from a clinical, epidemiological and economical perspective. Ticks, fleas and sandflies are manifest examples of arthropods with a significant importance as parasites themselves and as vectors of pathogenic microorganisms affecting humans and both domestic and wild mammals. An updated overview of the epidemiology and diagnosis of the principal arthropod-borne microorganisms pathogenic to companion animals, and with relevance for public health, will be presented during this symposium, focusing on vectors such as ticks, fleas and sandflies present in Latin America. Additionally, because most of the available methods for controlling arthropod vectors rely on the use of synthetic insecticides and acaricides, a major problem related to the emergence of multidrug resistance to these compounds has widely emerged worldwide. Perhaps this is the principal current limitation to overcome for the chemical control of parasites. Therefore, some topics about resistance of arthropod vectors to insecticides and acaricides are also included in this symposium.

Coordinator:	NAVA, Santiago
Speakers:	CICUTTIN, Gabriel LAMATTINA, Daniela VENZAL, José RECK, Jose TORRENTS, Jorgelina

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Ehrlichiosis and bartonellosis: Epidemiology and diagnosis in companion animals

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Ten thousand years ago humans domesticated the first animals, and the human-animal relationship has evolved from a material supply (food, shelter, mobility) to satisfying an emotional need. Most households worldwide have pets, the most frequent species being cats and dogs.

Ehrlichioses are tick-borne diseases that affect humans and animals. Canine monocytic ehrlichiosis (CME) is caused by *Ehrlichia canis* and transmitted by the tick *Rhipicephalus sanguineus* sensu lato. In dogs, it causes a multisystemic disease that can be acute, subclinical or chronic. The zoonotic potential of *E. canis* is considered low. Natural *E. canis* disease in cats is not fully elucidated. In 2013, clinical cases of CME were reported for the first time in Argentina. To date, its occurrence has been confirmed in practically the entire north-central of the country.

The genus *Bartonella* currently consists of more than 30 different species, and their number has been increasing rapidly in recent years. Approximately 15 species have been implicated in human disease. Cat scratch disease, caused by *Bartonella henselae*, is the most common pet-borne zoonosis worldwide. The reservoir of *B. henselae* is the cat, which usually presents a chronic infection (generally asymptomatic). Transmission occurs by scratch or bite, and by the cat flea *Ctenocephalides felis*. In dogs, *B. henselae* produces disease (with heart afection, among others) similar to those in humans. In the last decade, the study of the genus *Bartonella* in companion animals has begun in Argentina. *B. henselae* and *Bartonella vinsonii* subsp. berkhoffii in dogs and *B. henselae* and *Bartonella clarridgeiae* in cats have been reported.

Epidemiological aspects of canine visceral leishmaniasis in an endemic area of Argentina

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Canine Visceral Leishmaniasis (CVL) and Human Visceral Leishmaniasis (HVL) are among the vector-borne parasitic diseases with the greatest impact on public and veterinary health in the world, and are endemic in the province of Misiones, northeastern Argentina. The main urban reservoir in America and much of Europe for *Leishmania infantum* is the dog. The protozoan is transmitted in our continent mainly by the bite of sandflies of the species *Lutzomyia longipalpis* and also between dogs by vertical and venereal transmission. In addition to being a highly lethality global zoonosis, CVL is a disease that is difficult to manage due to its complexity and the wide variety of clinical manifestations, which range from asymptomatic dogs to severe symptoms. There are no preventive drugs against VL nor a vaccine in use for humans or canines that has demonstrated effectiveness as a prevention or control tool in collective health, and although the insect *Lu. longipalpis* is susceptible to household insecticides, its application has not shown effectiveness to interrupt transmission. There are insecticide repellent collars impregnated with pyrethroids for dogs, which anti-feeding effectiveness extends for periods of 6 to 12 months, but the high acquisition costs translate into little acceptance and adoption by dog owners in endemic areas. In turn, the low sensitivities and failures in timely diagnosis, added to the high rates of dispersion due to pet transit and trafficking, make it difficult to manage infected dogs. In Misiones, a series of studies were carried out in different locations, and the results of surveys, statistical models, and cartographic outputs were used to evaluate the prevalence, spatial distribution, and factors associated with CVL. These and other epidemiological aspects of CVL in an endemic area of Argentina will be addressed, presenting the main challenges for the prevention and control of this parasitosis.

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Canine Piroplasmosis: vectors, epidemiology and diagnosis

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Piroplasmosis are caused by apicomplexan protozoans of the order Piroplasmida. Currently this order includes the genera *Babesia*, *Cytauxzoon* and *Theileria*. They are hemoparasites transmitted by ticks (mainly from the family Ixodidae) to vertebrate hosts (mammals and birds). Worldwide, the most common piroplasmosis in dogs is babesiosis, caused by approximately eight species of *Babesia*. The another piroplasmosis in dogs is rangeliosis, caused by *Babesia vitalii* (=*Rangelia vitalii*) with a distribution exclusive to South America. The clinical babesiosis in dogs are characterized by fever, anemia, hemoglobinuria, jaundice, hepatomegaly, splenomegaly, and death in some cases, but asymptomatic infections are also common. The severity of clinical cases is mainly due to the species of *Babesia* involved as well as factors depending on the dog (age, immunodeficiency, and coinfection with other pathogens). In Latin America, the most frequent and distributed canine piroplasmosis is caused by *Babesia vogeli* and transmitted by *Rhipicephalus sanguineus* sensu lato. The pathogenicity of *B. vogeli* in adult dogs is mild or subclinical, although it can be severe in pups or in immunosuppressed and splenectomized dogs. In contrast, canine rangeliosis, popularly known as "nambi-uvú" or "bleeding ears", was reported only in Argentina, Brazil, Paraguay and Uruguay. *Babesia vitalii* is transmitted by *Amblyomma aureolatum* and infects endothelial cells in addition to erythrocytes and leukocytes of dogs and wild canids. Due to endothelial damage, severe thrombocytopenia results from platelet consumption, causing persistent bleeding and death of the animal. The remaining canine babesiosis mentioned for Latin America is caused by *Babesia gibsoni*. It is transmitted by *R. sanguineus* s.l. and *Haempahysalis* spp. In our region this babesiosis has few confirmed reports. In the diagnosis of canine piroplasmosis, epidemiology and clinical data are relevant. Traditionally, diagnosis is made by blood smear although IFAT, ELISA and PCR assays are frequently used.

Resistance to antiparasitic drugs for arthropod ectoparasites in Latin America: the case of cattle tick *Rhipicephalus microplus* in Brazil

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The cattle tick, *Rhipicephalus microplus*, causes severe economic losses to beef and dairy production in several countries. This species is distributed among subtropical areas with large cattle herds, such as Brazil, Argentina, Uruguay, Mexico, India and some African countries. This tick species has also a great importance as a vector, since *R. microplus* is associated with the transmission of pathogens, such as cattle babesiosis. Currently, the control of the cattle tick is based mostly on the use of chemical acaricides. In the face of that, there are emerging reports of tick populations multirresistant to chemical acaricides, especially in Latin America. In Brazil, our research group identify since 2014 tick populations resistant to all six chemical classes of acaricides available on the market. Since then, two major points become peremptory: to improve the diagnosis of acaricide resistance, and to validate strategies of tick control. Considering the results of more than six hundreds of routine assays based on the test of immersion of adult ticks (TIA), approximately 90% of samples were resistant to the most traditional acaricides in market, synthetic pyrethroids. The resistance reports are also increasing each year for other chemical classes, such phenylpyrazoles and macrocyclic lactones. On the other hand, we have been working on novel strategies of tick control, such the sustainable use of chemical acaricides and biological control. Recently, we have shown the feasibility of the generational control of cattle tick using six treatments/year rotating three different classes of acaricides even facing a multirresistant tick strain. Moreover, we also showed the viability of the use of fungi formulations to control *R. microplus* ticks under field conditions using cattle spray race apparatus. The set of results presents a great challenge in the control of cattle tick, but also brings novel tools to be considered.

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Current status of tick resistance to synthetic chemical acaricides in Argentina

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Rhipicephalus microplus is one of the most important hematophagous ectoparasites distributed in tropical and subtropical areas worldwide. The damage caused for direct action of ticks over cattle and the indirect effects as economic losses and sanitary consequence is the reason because the control is an important point in the livestock development. The control is mostly based in the use of synthetic chemical acaricides which has led in resistance strains. In Argentina currently there are multiresistant strains to all the active principles actives available on the market. Chronologically the resistance appears in our country in 1948 for arsenical products about a decade later organochlorines resistance was reported. Both chemicals groups must not be used at present because they were withdrawn from the market in 1969. Posteriorly, in 1977, resistance to synthetic pyrethroids appeared in Argentina. Its high efficacy and low toxicity in animals and man caused this resistance to spread in the distribution areas of the parasite. In this way, the implementation of other acaricides began and the appearance of resistance began to precipitate. In 1996, the appearance of resistance to organophosphates was described; hence, in 2013 it was documented for amitraz, 2018 for fipronil, 2020 for ivermectin and finally 2022 for fluazuron. Perform control strategies for *R. microplus* includes knowledge of its distribution, ecological and biological aspects, as well the application of diagnostic techniques that allow the effective detection of resistant strains. In conclusion, there are reports of *R. microplus* resistant to all the chemical groups used as acaricides in Argentina.

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Symposium "Four realities: Latin American regulations applied to genetically modified (GM) insects used in agricultural and livestock context"

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In the II Congress of the Latin American Society for Vector Ecology (LA SOVE), current topics of regional interest have been included such as regulations on GM insects. This incorporation highlights the advance of science along with the roles played by insects in agricultural and livestock production, not only those beneficial but also the negative ones (as pests and disease vectors). Recently, there has been an increase in research on GM insects obtained by Modern Biotechnology targeted to provide solutions for food production and improve the health of farm animals and crops. Worldwide, there are diverse regulatory frameworks applicable to these types of scientific developments, including GM insect pests and vectors focused on population control. With the purpose of showing different realities, four (4) Latin American (LA) countries were identified as representative candidates, namely, Mexico, Cuba, Brazil and Argentina. Disseminating information regarding different LA regulatory systems for GM insects that are used in agricultural and livestock context, is fundamental for the promotion and development of the scientific and technological sectors. It is important to point out the value of regulating activities that involve the use of GM insects, due to their potential impact on agricultural and livestock production. Sharing the different regulatory experiences is not only intended to promote and motivate the generation of new regulatory systems in other countries, but also to provide a space for exchange, debate and discussion about current regulatory systems. The four LA countries that are participating have a specific regulatory system for GM animals, which include GM insects used in an agricultural and livestock context. In all of them, two distinct phases of regulation are found: Confined activities (research) and release into the agroecosystem. Having an up-to-date regulatory system prepared to receive applications for these GM insect developments is considered valuable for scientific progress in LA. The inclusion of this topic in this Congress contributes to disseminate the existence and scope of the different regulatory frameworks applicable to GM insects that are used in agricultural and livestock context, in the LA entomological community.

Coordinator:	MÜHL, Marina
Speakers:	BOARI, Paulina DAGLI ZAIDAN, Maria L SUAREZ ROMERO, Marvis E CERVANTES, Marco A

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Argentine regulation applicable to insects obtained by modern biotechnology for use in agriculture, livestock and poultry

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The Argentine State regulates activities involving animal species modified by Modern Biotechnology that can be used in agriculture, livestock and poultry since 1991 through the Ministry of Agriculture, Livestock and Fisheries (MAGyP). In that year, the National Advisory Commission on Agricultural Biotechnology (CONABIA) was created. Since then, it has functioned within the scope of the Ministry with an advisory role. The constant advance of science has opened the door to novel technologies. As a result of this, at present, Modern Biotechnology includes not only ADN recombinant technologies but also new breeding techniques such as gene editing. As a consequence, the national regulatory framework has evolved to meet the changing context of science as regards national and international developments. In Argentina, the use of genetically modified (GM) animals, whether for experimentation or releasing to the agro-ecosystem, has to be authorized by the Secretary of Food, Bioeconomy and Regional Development from MAGyP. The CONABIA advises the Secretary on the convenience of granting the permission that enables the applicant to carry forward the activity. Nowadays, there is an increase in research on GM insects obtained by Modern Biotechnology with application in agriculture, livestock and poultry. In addition to this, the regulation of activities with GM animals in confinement, including insects, has its sights set fundamentally on production and farm animals. So, it is important to analyze the regulatory system by broadening the vision, in order to detect if it requires any adaptation for confined activities involving GM insects. For these reasons, a working group (WG) was formed specifically for the analysis of the regulation for the case of GM insects. The WG was a space for consultation, exchange and construction with regulators, researchers and developers from the public sector, with federal participation, about the research on GM insects with application in agriculture, livestock and poultry. The guiding objectives of this WG were to analyze the current regulation, its articles, regulations and application form and, if necessary, to propose adaptations for applications presenting research (confined activities) with this kind of insect. The goal of this talk is to explain the scope of the Argentine Regulation applicable to insects obtained by Modern Biotechnology. Moreover, its purpose is to communicate the channels available to ask whether a particular investigation carried out using these kinds of insects should be regulated or not. Internationally, Argentina aims to share its regulatory system and the process of revision and adaptation of the Argentine Regulation made by the WG, remarking the conclusions at which it arrived with the Latin-American entomology community.

Biosafety of genetically modified or genetically edited animals in Brazil: role of CTNBio, and its regulations

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The regulatory body responsible for the biosafety of genetically modified organisms in Brazil is the CTNBio, the National Technical Commission on Biosafety. The CTNBio is a commission linked to the Ministry of Science, Technology and Innovation of Brazil, and has been firstly created in 1995 through the Law 8974 of January 5th, 1995. In March 24th, 2005, the new Brazilian Biosafety Law 11.105 was sanctioned and repealed the Law 8974. The Law 11105 renewed the CTNBio, which is composed by 54 members (27 full members and 27 alternates) who meet monthly. The Law No. 11,105/2005 establishes safety standards and inspection mechanisms for construction, cultivation, production, handling, transport, transfer, import, export, storage, research, marketing, consumption, release into the environment and the disposal of genetically modified organisms - GMOs and their derivatives, having as guidelines the stimulus to scientific progress in the area of biosafety and biotechnology, the protection of human, animal and plant life and health, and the observance of the precautionary principle for the environment protection. Has as one of its basic principles the case-by-case analysis. All GMOs can be analyzed regarding their biosafety by the principles of this Law: plants, microorganisms, vaccines, gene therapies, and animals. CTNBio accredits laboratories that wish to work with GMOs, which must submit annual reports to that commission. In addition, it reviews containment research projects, planned environmental releases, and commercial releases. One of the most important definitions refers to the definition of the risk class of the organism and the laboratory or field conditions in which it has been manipulated. CTNBio has normative resolutions that specify some aspects of the Law. Normative Resolution number 34 provides for the classification of risks of Genetically Modified Organisms (GMO) and the levels of biosafety to be applied in activities and projects with GMOs and their derivatives in containment. Normative resolution number 32 provides for the rules for commercial release and monitoring of Genetically Modified animals and plants - GMOs and their derivatives of plant and animal origin. CTNBio does not analyze genetically edited organisms. Genetically edited animals and organisms are analyzed in accordance with Normative Resolution number 16, of January 15, 2018. By means of a consultation letter, the process and the characteristics of the organism are analyzed by CTNBio, which will decide whether or not the organism will be exempted than what is required by Law 11105.

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Application of biological safety authorization system in Cuba to work with Genetically Modified Animals (GMAs)

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Cuba in accordance with the agreements adopted as State party to the Convention on Biological Diversity as well as to the Cartagena Protocol establishes and supports the means to regulate, manage or control the risks derived from the GMAs confined use and release into the environment, included insects for agricultural use. In that sense the Biodiversity and Biosafety Department, as a regulator authority, attached to the Office of Regulation and Environmental Safety (ORSA, in spanish), and the Ministry of Science, Technology and the Environment (CITMA), has established a biological safety authorization system, which is legally based on Decree Law 190/99 "On Biological Safety" and CITMA´s Resolution 180/07 on "Regulation for the granting of Biological Safety authorizations. Having a Biological Safety Authorization System for work with genetically modified animals, has made it possible to control the risks that could arise from the testing phases with these organisms to releases into the environment, through regulatory mechanisms like authorizations and inspections based in risk assessment and risk management. The application of this mechanism in activities involved with genetically modified animals is the objective of our presentation.

The regulation of the Genetically Modified Organisms in Mexico

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In Mexico, the Law on Biosafety of Genetically Modified Organisms (LBOGM) regulate the Genetically Modified Organisms (GMOs) activities. This federal law was published in 2005 in the Official Journal of the Federation, as a national legal system derived from the agreements reached in the Cartagena Protocol on Biosafety to the Convention on Biological Diversity; therefore, the Law implements the generic provisions established in the Protocol. The objective of the LBOGM is to regulate activities for the Confined Use, environmental release, commercialization, importation, and exportation of GMOs; aiming to prevent, avoid or reduce the possible risks that these activities may cause to human health, the environment, biological diversity, as well as plant, animal, and aquacultural health. Within the framework of the LBOGM and its Regulations (RLBOGM), a GMO is considered to be any living being, with the exception of human beings, which has acquired a novel genetic combination through the specific use of modern biotechnology techniques. The LBOGM establishes three stages of release into the environment: experimental stage, pilot programme, and commercial stage, each with specific requirements and objectives. The Confined Use is defined as any activity where the genetic material of an organism is modified; or, when such modified organism, is cultivated, stored, used, processed, transported, marketed, destroyed, or eliminated; and, in order to effectively limit its contact with the population and the environment, physical barriers, or a combination of these with chemical or biological barriers, are used when carrying out such activities. For the intents of this Law, the confined use facilities area is not part of the environment. The risk assessment process focuses on the "step by step" and "case by case" principles.

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Symposium "Interaction between Science and Health Management"

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Resistance management requires vector surveillance, prevention, and control activities that are based on scientific knowledge and that are carried out by the health management system. Despite the various tools developed to date for pest control, the use of pesticides remains part of the control strategies aimed at vectors of human diseases. Given the evolution of resistance to pesticides in triatomines and culicids, it is extremely necessary to have new alternatives for their management. In this sense, one of the objectives of the Red Argentina de Vigilancia de la Resistencia a los Plaguicidas de uso en Salud Pública (RAReP, Argentine Network for the Surveillance of Resistance to Pesticides for Use in Public Health) scientific-technical committee is to evaluate alternative insecticide control strategies and promote their rational use, optimising control actions from the perspective of integrated pest management. In this Symposium, the RAReP will be presented in the scientific field, possible alternatives to insecticides will be proposed for an integrated management of insect vectors, such as biological control, and the problem of Chagas in the urban sphere will be addressed, a new scenario that has increased during the last years. For this, the Symposium will begin with an introduction by the moderator where the needs of establishing the Network, its objectives, its components and difficulties will be described, and will continue with the development of research work within the RAReP framework. Finally, the Symposium will have a discussion section where the speakers and the rest of the attendees will discuss what has been presented, including the need for extending this discussion between the scientific field and government management in all problems in which this interaction is necessary.

Coordinator:	MARTI, Gerardo
Speakers:	MANTECA ACOSTA, Mariana CARBAJAL DE LA FUENTE, Ana L ACHINELLY, Maria F STERKEL, Marcos

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Emergence of the Argentine Network for Surveillance of Resistance to Pesticides for Public Health Use (RAReP)

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Vector-borne diseases, such as Chagas disease, leishmaniasis and dengue, have insecticides as their main control tool to reduce insect vector populations. Although this strategy has been useful so far for their control, the indiscriminate use of pesticides has led to the emergence and spread of resistant populations of insect vector species of health relevance throughout the world, and particularly in our region. The implementation of integrated vector management, which reduces the use of pesticides to a minimum, requires urgent research and subsequent implementation of strategies for the monitoring and management of resistance. For this purpose, within the National Ministry of Health, the "Argentine Network of Surveillance of Resistance to Pesticides for Public Health Use" (RAReP in spanish) was created, which is coordinated by the National Center for Diagnosis and Research on Endemic Epidemics (CeNDIE-ANLIS Malbrán) and the Directorate for the Control of Vector-borne Diseases and has an interinstitutional and interministerial scientific and technical committee that includes agencies, represented by the National Ministry of Health, ANLIS-Malbrán, CONICET, UNLP, ANMAT, National Ministry of Environment and Sustainable Development and support from the Pan American Health Organization. The purpose of this presentation is to explain the functions and activities being carried out by the RAReP. Its objective is to monitor pesticide resistance in arthropod vectors of pathogenic agents, to optimize the effectiveness and epidemiological impact of vector control activities carried out by the National Ministry of Health, and to propose alternatives for the control of these diseases from an environmental health perspective. We consider that this type of interministerial, interinstitutional and multidisciplinary articulation is the way to achieve the development of effective strategies that mitigate pesticide resistance, promoting an efficient management of insect vectors at the national level, with a perspective focused on public and environmental health, according to the guidelines of the multisectoral and sustainable approach of the Sustainable Development Goals.

Urban infestation by triatomines an overlooked phenomena for Chagas disease

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Chagas is a complex and multidimensional socio-environmental health phenomenon, in which different components converge and interact. Historically, this disease has been associated with insect vectors found in the rural environment. However, in the American continent, we are currently facing a new paradigm, in which different scenarios allow maintaining the vectorial transmission of the parasite through triatomine populations that either enter dwellings intrusively or colonize urban environments. Records of scientific reports (1909-2020 period) available in the PubMed and LILACS search engines were retrieved, using three criteria according to the main triatomine genera with epidemiological importance and to the general scientific production on Chagas disease in urban contexts. The complete review of the documents considered in this work allowed establishing a basis about the occurrence of triatomines in urban areas, the infestation of dwellings and green spaces, the circulation of *T. cruzi* in urban areas, and the size of the urban agglomerates where these events occur. Our results show that the urban infestation in the Americas is associated with 18 species of triatomines with varying degrees of adaptation to urban areas. The diagnosis of *T. cruzi* infection revealed that the parasite is transmitted by 17 different species of triatomines. The results also showed that the spatial and ecological coverage of triatomine infestation and parasite infection extends across the continent from Argentina to southern USA and that urban infestation has been increasing in the last three decades. An important point to highlight is that at least 18 species of triatomines belonging to the genera *Panstrongylus*, *Rhodnius* and *Triatoma* occur in urban areas. Infestation of triatomine species occurs in all types of cities (small, medium and large, including megalopolises). In this oral presentation, we will also focus our attention on the occurrence of *Triatoma infestans* and *T. cruzi* in San Juan, Argentina. Urban Chagas represents a new challenge that adds a different edge to the problem of Chagas disease due to the particular characteristics of the way of life in urban agglomerates. This will require adaptations of the programs of control of vector populations in urban areas and interaction with the community and academy.

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Nematodes as a biological alternative for the control of disease vector insects

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The global use of traditional synthetic insecticides to control vector insects in recent decades, has caused environmental contamination of aquatic and terrestrial ecosystems and has also given rise to resistance to insecticides in many species such as mosquitoes and kissing bugs. Pathogenic and parasitoid nematodes cause mortality in insects being an alternative of control. Parasitism by three entomopathogenic nematodes strains (EPNs) *(Hetrorhabditis bacteriophora* strains and *Steinernema feltiae*) produced a reduction of 90% in survival of pyrethroid-resistant and non-resistant nymphs of *Triatoma infestans*. No significant differences were observed between pyrethroid-resistant and non-resistant *T. infestans* populations parasitized by EPNs. The lowest survival of *T. infestans* nymphs resulted from application of *H. bacteriophora* strains. Nymphs (N1-N5) and adults were susceptible to infection at a concentration of infective juveniles of 64 IJs per cm2. In mosquitoes, the mermithid *Strelkovimermis spiculatus* was widely studied. This nematode was isolated from natural populations of *Aedes albifasciatus* larvae in rainy environments of the province of Buenos Aires, Argentina in epizootics levels, and at enzootic levels in other species when environments remained flooded for prolonged periods of time. Infective stages of *S. spiculatus* were able to infect and kill larvae of *Ae. aegypti, Culex apicinus* and *Cx. pipiens* at field conditions. The evaluation in containers of 90 liters of capacity colonized naturally by these species caused levels of infection between 70-100% at a concentration of 100 J2/host. Mosquitoes larvae were observed parasitized by the nematode for approximately 10 days. We are currently working on field application techniques based on entomopathogenic nematodes (*H. bacteriophora* strains) and parasitoids (*S. spiculatus*) to control populations of the vectors *T. infestans* and *Ae. aegypti* respectively.

Repositioning of Nitisinone for the selective control of hematophagous arthropods

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Hematophagous arthropods incorporate many times their weight in blood during each meal. Since proteins represent more than 85% of blood dry weight, enormous amounts of amino acids are released during its digestion. Recently, we demonstrated that the inhibition of the tyrosine catabolism pathway causes the death of hematophagous arthropods after feeding due to the accumulation and precipitation of tyrosine. However, the inhibition of tyrosine catabolism does not affect organisms with other feeding habits. In addition to revealing a novel aspect of the evolution of hematophagy, the results indicate that this pathway may be a new target for the selective control of vectors without affecting non-hematophagous organisms. Inhibitors of tyrosine catabolism are currently used in medicine and agriculture as herbicides. Among the inhibitors that have been tested in different vectors, the most potent is Nitisinone (NTBC), an orphan drug currently used to treat a genetic disease called Tyrosinemia type I. NTBC is lethal to vectors when topically delivered, like pyrethroids, or when it is administered to the vertebrate host as an ectocide, similar to ivermectin. Therefore, we propose that NTBC could be repurposed as a new insecticide for the selective control of vectors.

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Symposium "Integrating Approaches for Vector Control"

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In recent decades, vector control has become a worldwide challenge due to the serious threat of vector-borne diseases (VBD) to human health and well-being. They are highly associated with urbanisation, travelling, climate change, resistance to traditional synthetic insecticides, and the evolution of pathogen strains, among others. The World Health Organization recommends and emphasises the importance of controlling these vectors using diverse methods. In this symposium different strategies will be discussed, both in experimental and real case scenarios, understanding that every approach has its advantages and disadvantages. These diverse strategies include knockdown insects, sterile insect release (both biological sterility through symbiotic bacteria and chemical sterility), physical (removal of breeding sites), and biological methods (*Bacillus thuringiensis* var. *israelensis* and copepods), that could be part of a management program integrated into a vector control strategy in order to create a sustainable and profitable control. Finally, the advantages and perspectives of VBD control in the Americas, as part of the Regional Entomology in Public Health Program and Vector Control of the Pan American Health Organization, will be discussed.

Coordinators:	MICIELI, María V PIMENTA, Paulo
Speakers:	PEREIRA, Marcos H LORENZO, Marcelo G XUE, Rui-De BECKER, Norbert BEZERRA, Haroldo

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Feeding behaviour of arthropod vectors

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Understanding basic biology/physiology is paramount for adequately designing control strategies. Hematophagy is an unusual feeding strategy among arthropods occurring in <1.6% phylum diversity. However, hematophagous arthropods possess great medical-veterinary relevance, acting as pathogen vectors of important vertebrate diseases. In general, during hematophagy, pathogens circulate between the vertebrate host and their vectors. In addition, ingested blood may represent the only nutrient source for some arthropods (e.g., triatomines, ticks) or as a nutritional stimulus for oocyte production (e.g., sandflies, mosquitoes). Therefore, differences in feeding performance directly affect vector competence and/or insect vector population dynamics. Despite a marked phylogenetic diversity among bloodsucking arthropods, adaptive convergences related to hematophagous behavior are observed. Among them are the morphological adaptations of mouthparts, including structures selected for perforating, penetrating, and anchoring mouthparts in the host skin, as well as the composition of saliva, which is rich in biomolecules capable of interfering with vertebrate physiology and helping with blood intake. During hematophagy two crucial spots of the vector–host interface are established. One is formed by the alimentary canal opening of arthropods ("functional mouth") and the host skin/endothelium. The other is created by the arthropod midgut environment and the host blood. The combination of RNAi-Induced Gene knockdown (RNAi) with intravital microscopy techniques has allowed evaluating triatomine genes (salivary and intestinal) for obtaining bloodmeal in a live host. Saliva labeling with fluorochrome Acridine Orange allowed observing the salivation pattern of hematophagous Hemiptera (*Rhodnius prolixus* and *Cimex lectularius)* during blood-feeding. In these ectoparasites salivation begins with the bite, continues until the end of the blood meal, and shows two distinct patterns. During the probing phase, salivation is abundant and continuous, whereas, during the engorgement phase, less saliva is released and in lower frequencies, mainly into the blood vessels. The insects ingest part of the released saliva along with the blood meal. Thus, salivary molecules can act together with the intestinal components when blood is ingested by the insects. Knockdown insects for salivary genes, the nitric oxide synthase (NOS), and the NO-carrying (nitrophorins), exhibited lower blood ingestion rate than control insects. Interestingly, a reduction of the expression of the *R. prolixus* CYP4EM1 (a cytochrome P450) gene, expressed majority in the salivary gland (> 100 times more than in other organs/tissues), did not affect the insect blood intake performance, but induced deleterious effects in nymphs exposed to sublethal doses of deltamethrin. The reduction in the expression of the intestinal thrombin inhibitor (Brasiliensin) of *Triatoma brasiliensis* by RNAi, led to a significant decrease in the amount of blood ingested by insects when fed in the live host when compared to controls. This blood meal size reduction probably is associated with the lower ability to maintain the high initial blood pumping frequency of Brasiliensin knockdown insects. Once, both effects were reversed when knockdown insects were fed on mice previously treated with a potent systemic anticoagulant (heparin).

Mom knows better: Egg-laying by female *Aedes aegypti* shapes the bacterial communities of breeding sites

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Aedes aegypti, the main vector of multiple arboviruses, is highly associated with human dwellings. Females exhibit an opportunistic oviposition behavior, seldomly laying eggs on natural containers, but rather distributing them among human-generated breeding sites. Bacterial communities associated with such sites, as well as the compositional shifts they undergo through the development of larval stages, have been described. Some bacteria can play a direct role in supporting the success of mosquito development. Additionally, exposure to different bacteria during larval phases can have an impact on life-history traits. Whether the larvae acquire symbionts from aquatic niches, or just require bacteria as food, is still debated. Based on these facts, we hypothesized that female *Ae. aegypti* shape the bacterial communities of breeding sites during oviposition as a form of niche construction to favor offspring fitness. Our study presents a series of experiments to address whether gravid females modify bacterial consortia present in larval habitats. For this, we first verified if females can mechanically transfer bacteria into culture media. As evidence of mechanical transmission was obtained, we then elaborated an experimental scheme to dissect effects from factors related to the act of oviposition and mosquito-egg-water interactions. The DNA samples obtained from breeding site water belonging to five treatments were subjected to amplicon-oriented sequencing to infer their bacterial community structure. Microbial ecology analyses revealed significant differences between treatments in terms of diversity. Particularly, between-treatment shifts in abundance profiles were detected, also showing that females induce a significant decrease in alpha diversity through oviposition. In addition, indicator species analysis pinpointed bacterial taxa with significant predicting values and fidelity coefficients for the samples in which single females laid eggs. Furthermore, we provide evidence regarding how one of these indicator taxa, *Elizabethkingia*, exerts a positive effect upon the development and fitness of mosquito larvae, thus suggesting that the developmental niche construction hypothesis may hold in this model.

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Program overview of Anastasia Mosquito Control District, St. Augustine, Florida, USA

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Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida, USA established in 1948. AMCD is an independent special district. AMCD has expended for many times since the 1960's and covers whole county (609 square miles) in 2002. Currently AMCD has 32 full time employees, 6-8 seasonal full time, 7-8 intern students, and 5-6 visiting scientists with $6-7 million budget annually and serves more than 300,000 population. AMCD programs includes customer service and providing service request, surveillance of arbovirus and mosquito populations, operation control by ground and aerial application, applied research, and education. Since 2015 AMCD has built a 18 acre complex with administration building, a trap room, taxonomic lab, bioassay lab, molecular lab, 3 insectaries (one under quarantine), 2 quarantined greenhouses, wind tunnel lab, droplet size detection lab with laser machine, a multi lab, 3 large outdoor enclosures, 24 field larvicide pools, animal house, helicopter hangar with 3 helicopters, large vehicle storage with 42 vehicles, mechanic shop, and 2 pesticide storages (1 for ground and another one for aerial), and a 400 m by 400 m field testing site. AMCD also built the disease vector education center (6,000 s.f.) and the sterile insect technology for mass rearing facility (6,000 s.f.) is under construction. AMCD is a recognized Good Laboratory Practice (GLP) and the Northeastern Florida Mosquito control, applied research and education center. The District operated under the Florida status chapter 388 and governed by 5 elected officers. The district has been recognized as an intern training site by the CDC Southeastern Center for Excellence in Vector-borne Diseases, one of ten training hub hosts by American Mosquito Control Association in 2017-2018, a mentor organization by the National Association of City and County Health Offices to train small programs in 2019 and 2020. The District staff received 16 state and national awards and authors and coauthors of more than 200 publications and book chapters, and received multi millions funds from federal, state, and private organizations in the past 15 years. Currently, AMCD has a strong applied research and education team with 5 Ph.D. scientists, 5 M.S. and 3 B.S. biological technicians and work on several grant-funded projects.

Implementation of new Techniques in the integrated Management programme against *Aedes albopictus* in Germany

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Over the last decades, the Asian Tiger Mosquito, *Aedes albopictus* (Diptera: Culicidae), has spread globally due to its great adaptive potential, rapid and easy means of transportation and the global warming supporting its thrive under moderate climate conditions. It even conquered temperate geographical regions, including Germany where it already established more than 20 reproducing populations mainly in South-West Germany, but also in the capital city of Berlin in the North. The steady progress of introduction and establishment poses a tremendous nuisance and risk to human health and well-being, since the mosquito is known for its high vector competence, including arboviruses such as the Dengue or Chikungunya virus. Therefore, physical and biological vector control strategies aim to maintain its populations at a minimum or even to eliminate them before mass development, to prevent nuisance and local arbovirus transmissions. The control strategy comprises three components or pillars: a) community participation (CP) based on the elimination or sanitation of breeding sites with the use of fizzy Bti-tablets (Culinex® Tab plus); b) Door-to-Door (DtD) control by trained staff in a two to three week interval applying high doses of a Bti-water-dispersible granular formulation (Vectobac® WG) aimed for a long-lasting killing effect of at least three weeks; and c) the Sterile Insect Technique (SIT) to eliminate remaining *Ae. albopictus* populations. This strategy led to a significant reduction of the *Ae. albopictus* populations by more than 95% and in some cases even to elimination. However, in the heavily infested areas, retreatments of Bti in a three-week interval are necessary which results in high costs for personnel. Therefore, we aim to include copepods in our control strategy to achieve a sustainable effect for many weeks or even months. Copepods (Crustacea) are already known to effectively kill and prey upon mosquito larvae and for their ability to evoke long-term suppression of larvae in combination with Bti. First laboratory and semi-field evaluations, concerning the predatory potential of native German cyclopoid copepods, namely the field-derived species *Megacyclops viridis* (*M. viridis*; Crustacea: Cyclopidae), revealed high predation efficiencies up to 80.0 to 100.0% upon first instar larvae of *Ae. albopictus*. It is evident that copepods are promising candidates as biocontrol agents against Tiger mosquitoes and should be further investigated, especially aiming on the mass rearing techniques and facilities to achieve their integration to the local vector control strategy, in order to create sustainable, cost-effective and eco-friendly mosquito control.

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Advances and Prospects for Vector Control of Vector-Borne Diseases in the Americas

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Despite efforts to control and/or eliminate them, vector-borne diseases (VBD) remain as a serious threat to health, well-being, and the economy in the Americas. Within the various strategies implemented to deal with VBD, surveillance and vector control play a predominant role, which is why they continue to concentrate a significant part of the efforts. However, various circumstances make it difficult for their results and achievements to be complete. Among the main ones, it is possible to mention: Global warming which, added to the adaptive capacities exhibited by most vector species, has expanded their distribution areas, and modified other aspects of their bionomics and eco-epidemiology. The sustained increase in the urbanization of human populations on the continent, with its implicit problems of inequality in access to public services. The phenomenon of resistance to insecticides in various vectors. The conceptual lag, the technical-operational weaknesses, and the need to strengthen the integration and involvement of sectors other than the health sector in VBD prevention and control actions. Given this reality, reviews focused on understanding the complexity of VBD transmission, identifying its multiple dimensions, and improving the efficiency of control programs through the introduction of novel approaches, framed in the concept of integrated vector management of vectors (IVM). In this context, the Americas region is implementing the *Plan of Action of Entomology and Vector Control 2018 - 2023* (CD56/11, PAHO 2018), which seeks to strengthen national surveillance and control of key vectors. This plan consists of innovative strategies that focus on the application of IVM, the evaluation and management of resistance to insecticides, as well as training and capacity building to improve entomological practice, and the evaluation and incorporation of new technologies to vector surveillance and control. In follow-up of its implementation process, permanent technical cooperation is provided to the countries of the Americas to establish mechanisms and ways of adapting the Plan of Action to the local entomo-epidemiological context. In this framework, the new *Aedes* control model stands out, whose stratification proposals allow for dynamic evaluations of risk scenarios to identify priorities that guide cost-effective interventions. The present and future of vector surveillance and control requires: a) better coordination of all partners and stakeholders; b) the revision and update of the training of specialists and technicians, as well as of the national policies and strategic frameworks; c) the use of new vector control tools and techniques; d) better entomological and epidemiological surveillance of IVM at all levels of the health system; and e) approaches adapted to local environmental, entomological and epidemiological conditions. This is synthesized in the challenge of developing and implementing new approaches based on comprehensive and integrated actions, under the premise of efficient and effective use of resources.

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DISCUSSION PANEL

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Discussion Panel "Challenges and perspectives to address some neglected diseases"

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Neglected infectious diseases (NIDs) including those that are vector borne, are a group of infectious diseases, many of them parasitic. They mainly affect human populations with limited access to healthcare services, whether in rural or urban areas. Currently, hundreds of thousands of people are affected by NIDs worldwide. Although great progress has been made in recent decades and currently global interest regarding NIDs has been increasing, their control and elimination are threatened by multiple factors. The goal of this meeting is to show the impact of NIDs by highlighting their main problems and challenges, and by encouraging an exchange and debate of the different experiences in our region. We will focus on some specific NIDs identifying the actors involved, the interface between decision-makers and academia, challenges, and perspectives. We will take the case of Colombia as a central axis of discussion, as well as medical-care experience with zoonotic diseases in one high-complexity public hospital in the Buenos Aires Province, Argentina. We will also talk about the difficulty of diagnosing Chagas in the city of San Juan, Argentina, and the complexity of this problem in the new urban scenario. In addition, we will share the experience of a group that works with the Chagas problem from an integrative and innovative perspective taking diverse educational, community and health-care contexts into account.

Coordinators:	CARBAJAL DE LA FUENTE, Ana L SALVÁ, Liliana
Speakers:	PARRA-HENAO, Gabriel MELI, Sergio A ANGELETTI, Virginia CASAS, Natalia CARBAJAL DE LA FUENTE, Ana L

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Addressing neglected tropical diseases. Actors involved, interface between decision-makers and academia, challenges and perspectives. The case of Colombia.

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Neglected tropical diseases are highly prevalent in the tropical and subtropical regions of the Americas. To address their promotion, prevention and control, there are international bodies such as the Pan American Health Organization. In turn, this international body has complete coordination and work with the ministries of health of the member countries and this joint work is expected to help prevent and reduce the occurrence of these neglected tropical diseases. In turn, within the countries there are research groups that belong to the academia and develop research with funding from the Ministry of Science and Technology, but there is not always coordination between the priorities of the Ministry of Health and the research developed by the academia. The objective of this talk is to discuss the advances and problems encountered in the interaction between regulatory agencies and academia for research in neglected tropical diseases and highlight that interaction of research developed by academia with the work priorities outlined by the Ministry of Health is not always complete. Academics should try to harmonize their research processes with the priorities outlined by the health authorities, in the case of Colombia, with the ten-year public health plan.

Neglected diseases: preventions and cautions. Is it all about their impact? A brief overview of Chagas and its presence in San Juan

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According to the Pan American Health Organization (PAHO) portal, neglected or forgotten diseases are a set of infectious diseases, many of them parasitic, that mainly affect the poorest populations and those with limited access to health services; especially those who live in remote rural areas and in slums. However, some of them already affect the fringes of the urban population in large cities, not necessarily considered poor or with limited access to health services. Such is the case with the problematic of Chagas which have urbanized its scenario since some time ago, either by vertical maternal-fetal transmission, or by vectorial transmission. This has been happening in San Juan Province, Argentina, in the last six years. Three acute cases of vectorial transmission appeared in high population density urban areas in San Juan, with medium-low incomes. In these areas were detected triatomine domestic infestation including some cases of high densities and parasitized insects. These cases share a common factor regarding their diagnosis, in all of them the disease was not suspected at first, and two of them had Romaña's sign and therefore were initially considered as an ophthalmological pathology. The correct Chagas diagnosis of these cases were effectively succeeded from one up to three months after its initial presentation. What was the cause of the initial wrong diagnosis? Probably the diagnosis was affected by the unfortunately spread current ideology of the clinical setting: "Chagas is an old disease that has already been overcome", or even worse "Chagas is a phantom disease or forgotten". We assume that this consideration may be based on the immediate impact it causes. Initially, Chagas has almost no symptoms or signs in its acute stage. Additionally, in its chronic development it manifests a low percentage (20-30%) demonstrable associated cardiac or digestive pathology in the infected patients. We will also discuss what represents the appearance of acute Chagas infection. We will ask ourselves: is not perhaps an indicator of sanitary carelessness as well? Will the medical-clinical health system be able to resolve this controversy? Meanwhile the threat remains latent.

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Neglected infectious diseases: experience and challenges in the daily clinical practice

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Neglected infectious disease are a group of parasitic, viral, fungal and bacterial zoonotic diseases of poverty that impose a devastating human, social and economic burden on more than 1 billion people worldwide. They have historically lacked adequate attention in international public health efforts, leading to insufficient prevention and treatment options. There is still a large gap in our knowledge of these diseases, epidemiological data are scarce and they are underreported. The objective is to describe the experience in the daily clinical practice of neglected infectious diseases in a public hospital in Buenos Aires province. Neglected infectious diseases represents a challenge in daily clinical practice. There are multiple barriers in relation to diagnosis, treatment and follow-up of patients. A multidisciplinary approach is required for a correct approach to these pathologies.

Addressing zoonotic diseases in Argentina

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In Argentina, zoonotic diseases represent a serious public health problem with high morbidity and mortality rates and a high economic cost for health services due to the high costs of hospitalization and treatment of people. Some of these events are considered neglected diseases by the World Health Organization. The main endemic zoonoses reported are: rabies, echinococcosis, trichinellosis, hantavirus, leptospirosis, argentine hemorrhagic fever, brucellosis, hemolytic uremic syndrome, among others. These are under programme within the Ministry of Health and all have their surveillance and control standards, which were recently updated in 2022. Part of the Ministry's budget is intended to the prevention and control of these communicable diseases in relation to the purchase of supplies such as vaccines, dewormers, antivenoms, as well as training, field activities and prevention activities. The approach of zoonotic diseases needs to be under the concept of "One Health", an integrative and unifying approach that aims to achieve optimal and sustainable health outcomes for people, animals, and the environment. In this sense, multiple sectors, disciplines and communities at all levels of society are mobilized to work together to tackle threats to health and ecosystems. This approach has many benefits but is not always simple to implement in daily practice. Some of the main barriers to overcome are the disconnect between science, policy and action, the different interests between sectors and the lack of shared resources. We can also speak of language and cultural barriers among disciplines and sectors. It is essential to identify and overcome the barriers to achieve control of these diseases by working in an interdisciplinary and intersectoral manner, with community empowerment.

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Approaching the Chagas problem from a comprehensive perspective

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Since 2011, we have been working within the framework of the Group "What are we talking about when we talk about Chagas?" (¿De qué hablamos cuando hablamos de Chagas?). We, the members of the group, are people with and without Chagas, coming from different spaces and with different backgrounds (university, research, teaching, communication, art, health care and independent work) and varied institutional membership. Our aim is to approach the Chagas problem from an innovative comprehensive perspective in different educational, health and community contexts and settings. We define Chagas as a complex socio-environmental health issue in which elements of a different nature -biomedical, epidemiological, sociocultural and political-economic- converge and interact with each other, like in a puzzle, where each piece makes sense when combined with the other ones to form the complete picture. In turn, instead of statically complementary pieces, the Chagas components combine with each other dynamically, like the colorful "beads/pieces" of a kaleidoscope. Thus, we represent the complexity of Chagas with the metaphor of a "*kaleidoscopic puzzle*", where all components interact and complete each other in a dynamic way. From this conceptual metaphor in permanent movement and updating, we carry out actions to propose and understand the Chagas matter with different strategies and resources, appealing to the multiplying role of communication and education in health promotion, prevention and awareness. Our goal is to build and promote ways of looking at, understanding and dealing with the Chagas problem through different lines of action: university extension activities and public communication of sciences, research, educational proposals and resources, activism, articulated work with various institutions and social organizations, advice and technical assistance to health organizations, etc. In this presentation we share our experience, also seeking to join the critical and situated reflection on the challenges that arise from the presence of vectors in the current regional context.

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CHAGAS

eIF3 subunit m regulates blood meal digestion in *Rhodnius prolixus* affecting ecdysis, reproduction and survival

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In triatomines, blood-feeding triggers many physiological processes, including post-embryonic development and reproduction. Different feeding habits, such as hematophagy, can shape gene functions to meet the challenges of each type of diet. A comparison of transcriptomic and proteomic data indicates that post-transcriptional regulation of gene expression is crucial in triatomines, so we evaluated the impact of silencing of the eukaryotic translation initiation factor 3 subunit m (eIF3m) in *Rhodnius prolixus* physiology. In this work we used insects to evaluate oviposition, eclosion, survival and ecdysis rate. We used RNAi to determine loss-of-function phenotypes, applying techniques like RNA isolation and complementary DNA (cDNA) synthesis, synthesis of double-stranded RNA (dsRNA), quantitative polymerase chain reaction (qPCR), haemoglobin quantification, protein content measurement in ovaries and immunohistochemistry. eIF3m knockdown in the blood-feeder *R. prolixus* caused a reduction in the digestion rate affecting the processes triggered by the blood meal, such as reproduction in females and ecdysis in the nymph. Because oogenesis was blocked, females were more resistant to starvation and survived as long as the males after a blood meal. In nymphs, eIF3m silencing caused premature death and absence of ecdysis. The phenotypes observed in *R. prolixus* are different from those previously described upon eIF3m knockdown in *D. melanogaster* and are similar to those described upon DR in other (non-hematophagous) organisms.

Vertebrates as a food source for triatomines (Hemiptera: Reduviidae) vectors of *Trypanosoma cruzi*, in Argentina

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Chagas disease is caused by the intracellular parasite *Trypanosoma cruzi*, where triatomines act as vectors, mammals as hosts, and vertebrates in general as reservoirs. Understanding the transmission cycles of *T. cruzi*, and therefore the relationship between the insect vector and the vertebrates that act as a food source, is crucial to design and implement effective control strategies around this problematic that mainly affects the countries of America. For this, an exhaustive bibliographical compilation of scientific works published between 1960 and 2022 was carried out, from various sources of information currently available. The aim of the current work was collecting, systematizing and updating the information on the association between triatomines and vertebrates. These associations were classified as direct when they were based on molecular analyses; and indirect when triatomines and vertebrates met together in time and space. In turn, all the countries on which the reports included the 15 species of triatomines distributed in Argentina were recorded. Vertebrates were classified according to their corresponding specific category. Around 215 papers were analyzed, of which approximately 47% presented data on the association studied. Also, 553 reports were obtained from the following countries: Argentina, Bolivia, Brazil, Chile, Colombia, Paraguay, Peru, Trinidad and Tobago, Uruguay and Venezuela. Regarding food sources, 10 orders of mammals were included (Artiodactyla, Carnivora, Chiroptera, Cingulata, Didelphimorphia, Lagomorpha, Perissodactyla, Pilosa, Primates and Rodentia), as well as birds, amphibians and reptiles. The largest number of reports on vertebrates associated with triatomines corresponded to peridomicile animals such as cats, dogs, chickens, goats, pigs, etc. The only order associated with 100% of the argentine triatomines species was Rodentia. At the same time, it is clear that associations based on molecular analyzes represent much more robust evidence than indirect associations. According to this review *Panstrongylus geniculatus*, *Triatoma infestans* and *Triatoma sordida* are the most generalist argentine species of triatomines in their diet, presenting 11 different taxa as food sources for each of these species, also these were the triatomine species with the highest number of reports. In contrast, only one type of food source for *Psammolestes coreodes* and *Triatoma breyeri* were recorded. The systematized information was used to create a database on the argentine triatomines species and the associated vertebrate species as a food source. This data is necessary to create co-occurrence maps based on the geographical distribution of the species of vectors and their food sources, allowing the identification of areas of eco-epidemiological importance relevant to the transmission cycle of *T. cruzi*, in which reservoirs, vectors and hosts interact. The results obtained highlight the importance of directing future studies towards wild cycles, on which the information available is scarce.

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Characterization of CYP4G Integument Genes in the Chagas Disease Vector, *Rhodnius prolixus*

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The heteropteran subfamily Triatominae is a group of hematophagous insects mostly found in Latin America. They are vectors of Chagas disease, a parasitic illness affecting 6 to 7 million people worldwide. Insect cuticle hydrocarbons are involved primarily in waterproofing the cuticle, but also participate in chemical communication and regulate the penetration of insecticides and microorganisms. The last step in insect hydrocarbon biosynthesis is carried out by an insect-specific cytochrome P450 of the 4G subfamily (CYP4G). Two genes (*CYP4G106* and *CYP4G107*) have been reported in the triatomines *Rhodnius prolixus* and *Triatoma infestans*. In this study, the molecular and functional characterization of both *R. prolixus* CYP4G genes has been carried out in order to elucidate their roles in HC biosynthesis. Measurement of gene expression by RT-qPCR shows that both genes are expressed almost exclusively in the integument and have an expression pattern dependent on the developmental stage and feeding status. Knockdown of both CYP4G genes by RNA interference (RNAi) significantly diminished HC amounts both in the cuticle and internal tissues, with a selective cutback of straight or methyl-branched chains depending on the gene silenced. Molecular docking analysis between both CYP4G enzymes and different aldehyde precursors predicted their preferred interaction with straight or methyl-branched chains. Thus, both CYP4G genes are involved in HC synthesis, albeit with different substrate specificity. Survival bioassays exposing the silenced insects to desiccation stress showed that *CYP4G107* is determinant for the waterproofing properties of the *R. prolixus* cuticle. Our work demonstrates that distinct CYP4Gs have different specificity to form straight or methyl-branched HCs, and also provides clues to explain such specificity on the basis of the enzyme-substrate interactions obtained by a docking analysis. Further studies are needed to discover additional functions of these genes, especially in those insects that express three or more of them. Also, as our results indicate that there is compensatory mechanism regulating the expression of these genes, additional research is needed to elucidate this issue.

Sublethal effects of a deltamethrin on feeding and defecation patterns of *Triatoma infestans*

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Triatoma infestans is the main vector of the Chagas disease in the Southern Cone of America. One of the main forms of transmission is the vectorial one, through infected feces of hematophagous insects of the Triatominae subfamily. The main way to reduce the incidence of the disease is the chemical control of the insect vector with pyrethroids in homes and peridomiciles. The complexity in the construction and materials of peridomiciliary structures prevent a uniform application of insecticides while leaving them exposed to environmental degradation. Thus, the surviving insects that live there will be exposed for a time to sublethal doses of the applied insecticide. Sublethal doses can have different effects on the biology of the insects. The objective of this work was to evaluate the effect of sublethal doses of deltamethrin on feeding and excretion/defecation patterns of *T. infestans.* The experiments were carried out using fourth and fifth instar nymphs of *T. infestans* that came from a deltamethrin-susceptible strain reared under controlled environmental conditions (26 ± 1 °C; 60 ± 10%; 12:12 h). We worked with three experimental groups: exposure to a sublethal dose of deltamethrin, exposure to acetone (control 1) and no exposure (control 2). The insecticide and acetone were applied to fourth instar nymphs with 10-20 days old and fasted since the last molt. The feeding and defecation patterns was determined on fifth instar nymphs with 15–20 days old and fasted since the last molt. Each group was fed ad libitum and their feeding behavior and defecations were registered during and after feeding. Generalized linear model, two-way ANOVA with measures repeated over time and G-test of independence were used according to the dependent variable analyzed. In the feeding variables significant differences were observed for the number of feeding attempts and the number of feeding attempts with intake. In both variables, the acetone treatment showed significantly more attempts than the control without exposure and exposure to deltamethrin. However, these differences did not affect the size of the meal or the weight gain after feeding. On the other hand, no significant differences were observed between treatments for any of the defecation variables. Finally, treatment with deltamethrin showed the highest index of defecation (a measure of the number of parasite release events through feces that would occur in a given time) at the most relevant times for vector transmission; that is, during feeding and at 5 and 10 minutes post-feeding. Thus, this work suggests that a single exposure of *T. infestans* to a sublethal dose of deltamethrin in one stage could increase the probability of transmission of *T. cruzi* in the next stage.

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The *in vivo* treatment with the plant urease "Jack Bean Urease" impaired reproduction in females of *Rhodnius prolixus* (Hemiptera: Reduviidae)

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Ureases are enzymes that catalyze the hydrolysis of urea to carbon dioxide and ammonia. In recent decades, it has been postulated that plant ureases are also defense proteins against phytophagous insect species with potential biotechnological application. Previous reports demonstrated that the injection of "Jack Bean Urease" (JBU), the major isoform of urease from the legume *Canavalia ensiformis*, into the hemocele of triatomine insects, resulted in several toxic effects including activation of the immune response. Although the insecticidal effect of JBU was described several years ago, many aspects of its mechanism of action as well as the target organs remains largely uncharacterized. In particular, the effects of JBU on the female reproductive system and the consequences of sublethal doses have not been studied. In this work, we employed the Chagas disease vector *Rhodnius prolixus* as a model to study the effects of JBU on survival, ovarian development and oviposition of females. For the experiments, insects were injected with different doses of JBU in phosphate buffer. Control insects were treated with the same volume of vehicle. The results show for the first time that a sublethal dose of JBU impaired different reproductive parameters of *R. prolixus* females. All doses tested (0.01, 0.025 and 0.05 µg JBU/mg body weight) significantly decreased the number of eggs and the highest dose tested of 0.05 µg/mg delayed the onset of oviposition and hatching. However, only the dose of 0.01 µg JBU/mg did not cause insect mortality. Surprisingly, this sublethal dose that decreased the number of eggs resulted in increased insect longevity. Ovarian development of JBU-treated females was markedly delayed compared to controls. The ovaries exhibited ovarioles with atretic terminal follicles, which by high-resolution light microscopy showed cellular disorganization and vacuolization. Transmission electron microscopy displayed vacuolization and swelling of the nuclear membrane and mitochondria, being these findings compatible with cell death by necrosis. The reported findings are particularly relevant considering that reproduction and the achievement of a successful oogenesis are of key importance in the population dynamics of insect vector and pest species.

Phenotypic variability in the wing dimorphic species *Triatoma guasayana* in traits related to flight dispersion

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Triatoma guasayana is considered an emerging Chagas disease vector in the Southern Cone of South America. The presence of a population with brachypterous individuals, in which both wings are reduced, has recently been reported for this species. We aimed to understand if flight-related traits varied across populations, if these traits could explain the lack of flight dispersal in the brachypterous population, and if flight-related traits are associated with geographic and/or climatic variation. The study involved 66 males from 10 populations. We used digital images of wing, head and pronotum to estimate linear and geometric morphometric variables. We analysed variation in size and shape using one-way ANOVA and canonical variate analysis (CVA), respectively. We used Mantel tests to study the relationship between morphometric and geographic distances and the association between size measurements using Pearson's correlation. We explored covariation between size and shape variables using partial least square analyses (PLS). We tested the association of geographic and climatic variables with size measurements using linear regression analyses. We performed PLS analyses for shape measurements. Wing size differed significantly across populations. The CVA showed that wing shape of the brachypterous population is well discriminated from wing shape of the other populations. There was a positive and significant association between wing shape and geographic distances. The brachypterous population exhibited significantly larger heads than the other populations. As for wing shape, the head shape of the brachypterous population was well discriminated from the rest of the populations. Pronotum width did not show significant differences across populations. Geographic and climatic factors were associated with size and shape of both wing and head, but not with pronotum width. Most traits related to flight dispersal varied across populations. Wing and head shape were the best markers of the condition of lack of flight dispersal of the brachypterous population. Head measurements also varied in accordance with this condition. Geographic and climatic variables were associated with most of the flight-related traits.

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How is the phenotype of antennae, wings, and heads modified in *Triatoma infestans* resistants to deltamethrin?

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Triatoma infestans is an important vector of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. This insect represents a serious public health problem in Argentina due to the high density populations that develop in domestic and peridomestic environments in rural areas and, recently, in urban areas. Chemical control through spraying with pyrethroid insecticides is the only method to reduce populations of *T. infestans* in endemic areas. Despite this, resistance to pyrethroid insecticides is a problem in some localities in Argentina and Bolivia, where control actions with pyrethroid insecticides are ineffective. The genetic changes that determine resistance mechanisms to insecticides in an individual can have pleiotropic effects on physiological processes, causing changes that can be observed as different morphotypes. The objective of this work was to estimate the possible morphometric changes of wings, heads and antennal phenotype of males and females of *T. infestans* resistant to pyrethroids, evaluating the implication of the changes of the different structures in the adaptive processes of this insect. Thirty-six resistant (RR) and 58 susceptible (SS) adults (35 males and 59 females) from Chaco (Argentina) and raised in the laboratory (first and second generation) were analyzed. In these adults, the variables of size and conformation of heads and wings were analyzed, and the antennal *sensilla* of the three distal segments of the antenna were quantified. The results indicate that the RR insects suffer a phenotypic simplification compared to the SS. The antennae of RR females showed a significant decrease in the number of chemo- and mechano-*sensilla*. Antennae from RR males did not show this differentiation compared to SS. The wings were shorter and thinner than those susceptible for both sexes. In heads, the changes were less marked although a smaller distance between eyes is observed in RR. Regarding the fluctuating asymmetry, the RR ones showed more asymmetry in the shape of wings and heads than the SS. This result indicates greater developmental stress and instability associated with resistance to deltamethrin. The changes in wings, heads, and antennae suggest a lower sensory capacity and a lower potential for dispersal by flight in resistant insects. Although resistant insects have an adaptive advantage over susceptible ones in the presence of the insecticide, the resistance may imply a cost that reduces the energy devoted to various physiological processes or the modification of their phenotype with adaptive consequences in the environment without insecticide.

Characterization of the sensory gene repertoire of *Triatoma infestans* and the effect of blood ingestion on its antennal expression

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Currently, *Triatoma infestans* is the main vector of Chagas disease in Argentina, Paraguay, and Bolivia. As many *T. infestans* populations present insecticide resistance, directly impacting control campaigns, alternative control methods, like those based on behavioral manipulation are needed. Bug nutritional and developmental status modulate bug responsiveness to host-related sensory cues. In order to understand the molecular bases of this modulation, we sequenced the antennal transcriptome of *T. infestans* and compared the gene expression profiles between unfed and fed insects. For this, 6 RNA replicates *per* condition were used for sequencing (150 bp paired-end reads and 20 million reads/library). A *de novo* assembly was obtained using Trinity and DEseq2 was used to identify differentially expressed transcripts. The sensory repertoire of *T. infestans* was identified and compared to those of other hemipterans. *Triatoma infestans* has 127 ORs, 38 IRs, 11 GRs, 43 OBPs, 20 CSPs, 17 TRPs, 13 PPKs, 3 SNMPs, and 3 ammonium transporters. Six OR linages were expanded in *T. infestans* and *R. prolixus*, as well as the IR75 subfamily when compared to other hemipterans. The GR repertoire (11 transcripts) seems to be reduced in *T. infestans* compared to *R. prolixus* (28) and other hemipterans. However, this preliminary set of GRs is likely restricted due to the low antennal expression of gustatory receptors. The antennal expression of 2,122 transcripts was significantly modified after blood ingestion, including 41 sensory-related genes (9 were up-regulated and 32 were down-regulated). This work has allowed identifying gene candidates that could mediate host-seeking behavior in triatomines, which makes them potential targets to develop future behavioral manipulation strategies.

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Molecular and Functional Characterization of FASNs Genes in the Chagas disease vector, *Rhodnius prolixus*

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Fatty acid synthase is a multifunctional enzyme involved in the formation of fatty acids. Despite the role of fatty acids in cell signalling and energy metabolism, and as precursors to pheromones and hydrocarbons that waterproof the cuticle, the insect fatty acid synthases have been scarcely studied. Here we perform the molecular characterization of three fatty acid synthase genes (fatty acid synthase RPRC000123, RPRC000269 and RPRC002909) in the Chagas disease vector, *Rhodnius prolixus*. Gene expression screening by reverse transcription quantitative PCR showed that RPRC000123 and RPRC002909 are expressed almost exclusively in the integument tissue whilst RPRC000269 is mostly expressed in the fat body and also in several body organs. Phylogenetic analysis, together with gene expression results, showed that RPRC000269, RPRC002909 and RPRC000123 are orthologues of *Drosophila melanogaster* fatty acid synthase 1 (FASN1), FASN2 and FASN3 genes, respectively. After RNA interference-mediated knockdown of RPRC000123, insects died immediately after moulting to the next developmental stage. However, mortality was prevented by placing the insects under saturated humidity conditions, suggesting that dehydration might play a role in the insects' death. Lipid analyses in RPRC000123-silenced insects showed reduced amounts of integument fatty acids and methyl-branched hydrocarbons, compared to controls. These data support an important role for FASN3 in the biosynthesis of the precursors to hydrocarbons that waterproof the insect cuticle.

Involvement of lipophorin in the immune response of *Rhodnius prolixus* (Hemiptera: Reduviidae): challenge with the plant-derived entomotoxin jaburetox

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Lipophorin is the main lipoprotein circulating in insects. One of it its most thoroughly characterized function is the transport and transfer of lipids among target tissues. However, lipophorin also participates in insect immunity processes, changing the expression levels in response to pathogen challenges and neutralizing toxins in the hemolymph. On the other hand, ureases (urea amidohydrolases, E.C. 3.5.1.5.) are enzymes that catalyze the breakdown of urea in carbon dioxide and ammonia. In plants, besides generating bioavailable nitrogen, ureases fulfill a role in defense against phytophagous insects, thus exhibiting biotechnological potential for the rational control of pest species. Previous works in triatomines reported that the treatment with jaburetox, a recombinant peptide derived of the leguminous *Canavalia ensiformis*, induced toxic effects and the activation of the immune response. Even though the insecticidal effect of jaburetox was reported 15 years ago, several aspects of its mechanism of action are unknown. In this work, we employed the Chagas disease vector *Rhodnius prolixus* as a model to study the participation of lipophorin in the jaburetox-induced immune response. Analytical ultracentrifugation assays were conducted in order to assess lipophorin-jaburetox interaction *in vitro*. At 6 h after injection, lipophorin expression in the fat body, as well as its circulating levels in the hemolymph, were evaluated by biochemical, molecular and cellular approaches. The sedimentation rate obtained by analytical ultracentrifugation assays indicated a direct binding between lipophorin and jaburetox, reinforcing the implication of this mechanism of peptide detoxification *in vivo*. The results also show that, under our experimental conditions, treatment with jaburetox did not induce significant modifications neither in the expression of lipophorin in the fat body, nor in its circulating levels in the hemolymph. However, the injection of jaburetox impaired the association of lipophorin with the hemocytes, the main cells involved in insect immunity. Taken together, these findings support the involvement of lipophorin in the jaburetox-induced immune response of *R. prolixus*, providing valuable information to unravel the mechanisms of action of this toxin.

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Use of colorimetric analysis to study the origin of reinfestant *T. infestans* in a rural community of the Paraguayan Chaco

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One of the great purposes related to *Triatoma infestans* control, the main vector of Chagas disease in the Gran Chaco region, is to know the origin of reinfesting individuals found in domestic habitats after the chemical control actions carried out for its elimination. Among others, factors that hinder *T. infestans* control in this region are the recurrent houses reinfestation due to failures in coverage or in pyrethroids application, and the presence of reinfesting *T. infestans* with probable sylvatic origin. The peculiarity of individuals collected in sylvatic environments is that they are mostly melanic individuals with a darker appearance than the non-melanic forms commonly collected in (peri)domestic environments. In a previous study done in this area, there were no differences in wing size of domestic and sylvatic populations and 53% of post-intervention individuals were allocated to the sylvatic group. Here we analyze the potential origin of reinfestant *T. infestans* in 12 de Junio, an indigenous community from the Central Chaco in Paraguay, using a colorimetric quantification analysis. First, an entomological survey for searching domestic triatomines in (peri)domestic environments of all houses of the study area was conducted (prespraying period) before residual insecticide spraying campaign. Then an entomological follow-up was conducted between 3 and 12 months (postspraying period). The collection of sylvatic *T. infestans* was carried out around 12 de Junio locality. Color was quantified from image data of *T. infestans* females. We used the right side of connexivum using a homologous rectangle of 6 x 64 mm. We analyzed a total of 32 females from prespraying, 21 from postspraying and 10 from the sylvatic environment. The colorimetric analysis was performed using the software Image Color Summarizer 0.76. A K-means clustering analysis was used to define the number of colorimetric groups present in the prespraying and postspraying periods and in the sylvatic environment. The optimal number of clusters for all the data set was determined using the elbow and average silhouette methods. Results showed that elbow and average silhouette methods defined two colorimetric groups, melanic and non-melanic. All individuals from for the prespraying period (100%) were assigned to the non-melanic group, 58% of the individuals from the postraying period were assigned to the non-melanic group while only 40% of sylvatic individuals were assigned to this group. These results provide new evidence that sylvatic and domestic populations could be highly related to each other in the Paraguayan Chaco and that sylvatic *T. infestans* populations could be the source of new individuals in the processes of (peri)domestic reinfestations.

Calcium signalling on the basal activity of dorsal vessel in *Rhodnius prolixus*: an approach to the regulatory pathways in unfed insects

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Hemolymph recirculation in the Chagas disease vector *Rhodnius prolixus,* is facilitated by the coordinated activity of the dorsal vessel, which moves the fluid in a postero-anterior direction, and the anterior midgut redirecting hemolymph antero-posteriorly. During the post-prandial diuresis process, the frequency of contraction of both organs increases, stimulated by serotonin and allatropin. Furthermore, allatostatin-C antagonizes the effect of allatotropin. Although, all these messengers act through G protein-coupled receptors (GPCRs), little is known about the transductional signaling, and also about the relevance of Ca2+ as a muscle contraction regulator. The goal of this work is to analyze the basal regulation of the dorsal vessel, focusing in the importance of Ca2+. Physiological experiments were performed on unfed male insects. Each individual was placed under the stereoscopic microscope. The wings were moved, allowing aorta visualizing through the translucent dorsal cuticula. All treatments were applied throughout a little incision in the abdomen. As a first approach to understand the relevance of Calcium on the mechanism of contraction of dorsal vessel, two different chelators were assayed; EDTA (extracellular) and BAPTA-AM (intracellular chelator). To understand the ways used to increase cytosolic calcium level, ion channels blockers were used: Nifedipine (voltage gated calcium channels), and Ryanodine (Ryanodine receptors). Furthermore, to assay the probable involvement of signals coming from GPCRs, we assayed U73122, an inhibitor of phospholipase-C, Xestospongine-C, an inhibitor of IP3 receptor (IP3R) and SCH-202676, an allosteric modulator of GPCRs. Results are expressed as number of contractions/min and were analyzed by one way ANOVA. Single comparisons were tested by LSD. BAPTA-AM treatment diminished the dorsal vessel activity, showing that an increase of cytosolic calcium is needed for contraction. Similarly, treatment with EDTA completely abolished the activity suggesting that an extracellular source of Ca2+ is involved. To test the probable involvement of voltage-dependent calcium channels, insects were treated with Nifedipine, causing the frequency decrease, suggesting the relevance of extracellular Ca2+. The blockade of Ryanodine receptors also diminished the frequency. When IP3 receptors were blocked, a transient decrease of the activity was evident suggesting that GPCR mediated signals are also involved. Interestingly, the blockage of GPCRs by SCH-202676 caused the basal frequency increases, showing that it would be also regulated by an inhibitory pathway. When phospholipase-C was blocked the basal frequency was not altered. To ensure that U73122 blocked the activity of the enzyme, experiment on insects during post-prandial diuresis, when the frequency of the dorsal vessel is highest, were performed. The frequency was significantly diminished, suggesting that the dose used is appropriated. Finally, the results suggest that the dorsal vessel frequency during basal conditions depends on calcium modulation but, as in fed insects, the basal activity regulation also involves the of GPCRs signaling pathways.

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Basal regulation of dorsal vessel activity in *Rhodnius prolixus*: A probable myogenic rhythm regulated by peptidic signals

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Serotonin and Allatotropin act as cardioactive messengers increasing the post-feeding activity of the dorsal vessel in *Rhodnius prolixus.* Acting together, they increase the frequency of contractions of it, which is essential to facilitate the process of post-prandial diuresis. Interestingly when Allatotropin is applied to unfed individuals in vivo it has not activity, depending on the presence of serotonin to cause its stimulatory effect. We have also proved that Allatostatin-C has an antagonistic effect, diminishing the increase caused by Allatotropin. In a related study we show that the blockage of IP3R caused a transient but significant decrease of the dorsal vessel frequency suggesting that basal activity depends on a GPCR mediated signal. Interestingly, treatment with an allosteric modulator of GPCRs induced a stimulatory effect, suggesting that stimulatory but also inhibitory signals are involved in the maintenance of the basal activity of the dorsal vessel. The goal of this study is to analyze the integrative physiological way by which DV activity is modulated during basal conditions in *R. prolixus*. Physiological experiments were performed on unfed males. Each individual was maintained under the stereoscopic microscope after wings were removed, allowing aorta visualizing through the translucent dorsal cuticula. Treatments were applied throughout a little abdominal incision. Dorsal vessel activity is expressed as number of contractions/min. Statistical differences were tested by One Way ANOVA. To analyze a probable involvement of Allatotropin and Allatostatin-C on the basal activity modulation we tested the effect of Allatotropin and Allatostatin-C peptides and Allatostatin-C antisera in vivo. We also tested Allatotropin activity on an ex vivo dorsal vessel preparation. On the contrary to observed in vivo, preliminary results obtained by the ex vivo treatment of the dorsal vessel with Allatotropin show that the peptide is able to induce contractions, suggesting that the effect of Allatotropin in vivo depends on other signals that could be inhibiting its activity. As we previously have shown Allatostatin-C antagonizes the Allatotropin cardioaceleratory effect both after treatment with serotonin and during post-prandial diuresis. We checked now the probable effect of Allatostatin-C as an inhibitor of Allatotropin during basal conditions. We assayed both, the response of dorsal vessel to an Allatostatin-C antisera alone, and to the antisera applied together with Allatotropin. The results show that, whilst the antisera doesn´t produce any effect on the basal frequency of the dorsal vessel, the in vivo treatment with Allatotropin plus the Allatostatin-C antisera produces an increase of the frequency suggesting that, Allatostatin-C could be negatively modulating the effect of Allatotropin. Taking together, our results suggest that under basal conditions, the myogenic activity that ensure the minimum necessary activity of the dorsal vessel, could be modulated by signals involving Allatotropin and Allatostatin-C peptides.

Chemosensory proteins in *Triatoma infestans*: gene annotation and comparative analysis across hemipterans

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Triatoma infestans is the main vector of *Trypanosoma cruzi*, the causative agent of Chagas disease in the southern cone. Resistance to pyrethroid insecticides reported in *T. infestans* populations could be one of the main causes of the persistence of vectorial transmission in the Argentinean Gran Chaco ecoregion. Chemosensory proteins (CSPs) are small soluble proteins from arthropods, with a studied role in olfaction. Recent evidence points to a role of these proteins in insecticide susceptibility and resistance. Accordingly, previous results from our group showed that some members of *T. infestans* CSP family are modulated four hours after an intoxication with deltamethrin. The main goal of this work was to annotate CSPs encoding-genes in an available genome on *T. infestans*, and to perform comparisons of CSP gene family across hemipteran species. The genomic regions where CSPs are encoded were identified through BLAST searches, and the gene models were predicted using Fgenesh+ tool. The genomic position of the identified models was used to identify CSP gene clusters in the species, which could indicate a common gene regulation. A maximum-likelihood phylogenetic analysis was performed including these proteins along with the previously identified CSP sequences from other hemipteran insects such as the triatomines *Triatoma dimidiata*, *Triatoma pallidipennis* and *Rhodnius prolixus* and the pentatomid *Nezara viridula*. Moreover, the genomes of the cimicid insect *Cimex lectularius,* the pentatomid *Halyomorpha halys*, the delphacidae *Nilaparvata lugens*, and the anthocoridae *Orius laevigatus* were analyzed. The phylogenetic analysis revealed a conservation of some CSP genes throughout the Hemiptera evolution, while others seem to be specific for some families or genus. The results presented here provide valuable information on the evolution of this gene family. The comparison among species with different feeding habits and/or environmental niches could be useful to hypothesize about CSP genes involved in detoxification and/or insecticide resistance in harmful insect species.

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Can infection with *Trypanosoma cruzi* modify the toxicological response of *Triatoma infestans* susceptible and resistant to deltamethrin?

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Resistance to insecticides in *Triatoma infestans* is a problem that conditions one of the main control strategies for Chagas disease. However, the impact of the interaction between susceptibility/resistance of insects to insecticide and infection with *Trypanosoma cruzi* is unknown and could be diverse if the ecological interactions between the vector and the parasite are considered. In this framework, it is important to incorporate the insect-insecticide interaction in the study of the biological costs in natural interactions between *T. infestans-T. cruzi*. Nevertheless, there are no studies that have addressed the toxicological processes of triatomines from the parasite-vector perspective. The objective of this study was to determine the effects of infection with *T. cruzi* on susceptibility and resistance to deltamethrin in *T. infestans*. Insects from strains of *T. infestans* susceptible and resistant to deltamethrin were used for the tests. Infection by *T. cruzi* was induced in third instar nymphs. We worked with four experimental groups: susceptible uninfected and infected and resistant uninfected and infected with *T. cruzi*. Test to determinate deltamethrin susceptibility was performed by topical application bioassay on fourth instar nymphs, according to WHO protocol. Mortality was evaluated at 24-48-72 h post-exposure. From the dose-response curves, the LD_{50} and 95% CI were estimated for each experimental group. Comparisons of LD_{50} between the experimental groups were made using the Lethal Dose Ratio (LDR). The infected susceptible insects used as controls (exposed to acetone) died in a higher percentage than that accepted for this type of assay, so neither the LD_{50} nor the LDR could be calculated for this group. However, the high mortality in these controls and the higher mortality at the lower doses of deltamethrin in infected susceptible insects compared to mortality in uninfected susceptible insects suggest that the infection affected the overall susceptibility of *T. infestans* to exogenous toxic compounds. The LD_{50s} per insect and per unit body weight did not show significant differences between infected and uninfected resistant insects, and showed significant differences between these and uninfected susceptible insects, indicating that the two resistant groups have a similarly high resistance to deltamethrin. The infection does not modify the levels of resistance to deltamethrin, at least when these are very high, and increases the natural susceptibility of these insects to exogenous toxicants.

Presence of diverse discrete typing units of *Trypanosoma cruzi*, in Argentina

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Trypanosoma cruzi, the causative agent of Chagas disease, presents wide genetic diversity. Currently, six discrete typing units (DTUs), named TcI to TcVI, and a seventh one called TcBat are used for strain typing. These DTUs are defined as sets of populations that are genetically more related to each other than to any other evolutionary unit. The aim of this work is to survey the information available on the different DTUs of *T. cruzi* present in Argentina. The systematic review of the information was carried out mainly from bibliographic data and from the database of triatomines distributed in Argentina, developed by the CEPAVE Triatomine Laboratory and uploaded on the GBIF platform. Data were extracted from the species of triatomines and mammals where the presence of DTU of *T. cruz*i was found. In addition, information about the temporal and geographic framework of the study -including country/province/locality and/or geographic coordinates-, the diagnostic method, the type of biological sample and the number of infected individuals was collected. About 366 scientific works were examined between the years 1935 and 2017, of which 44 of them mention *T. cruzi* in their studies, all made in Argentina. Using optical microscopy as a methodology, infection by *T. cruzi* was not found in 20.45% of the cases analyzed. On the other hand, 22.72% of the works showed positive infection results in terms of seroprevalence or percentage, while 11.36% did not mention the methodology used to arrive at the positive infection result. Finally, the presence of the different DTUs was determined in 11.36% of the total works. The mixed infections were the most frequent with a 45.75%, TcVI with a 17.7 %, TcI with a 14.88%, TcV with a 11,45%, and TcII and TcIII with a 2.85% each. Most of these reports were found in the provinces of Chaco, Santiago del Estero and Tucuman. The vector species infected with the diverse DTUs were *Triatoma infestans* with 70.83%, followed by *Triatoma sordida* and *Triatoma eratyrusiformes* with 12.5% and 16.66%, respectively. The information found in wild mammals was scarce, finding mostly individuals included in the orders Carnivora, Rodentia, Cingulata and Didelphimorphia. This information is essential to understand the epidemiology of Chagas disease and so that through spatial analysis found areas of higher vectorial risk, a fundamental tool for decision makers on this problem. The need to expand the information on the presence of the several DTUs is crucial, since the use of data such as prevalence or percentages of infection cannot be used to produce these spatial patterns of risk of vector-borne diseases.

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Evaluation of differentially expressed genes in the brain and fat body of *Rhodnius prolixus* infected with *Trypanosoma rangeli*

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Triatomines are hematophagous insects which transmit *Trypanosoma cruzi*, the etiological agent of Chagas disease. Species of *Rhodnius* genus can also transmit *Trypanosoma rangeli*, a parasite that infect, but does not cause disease to humans. The physiological alterations that occur during trypanosome-triatomine interaction have been studied in different triatomine species, However, is crucial to understand the molecular mechanisms that modulate the interaction processes between triatomine and vector, especially in the association *T. rangeli-Rhodnius* once this combination causes strong effects on the insect. In this study we conducted a functional genomic study based on the analysis of the differential expression of *Rhodnius prolixus* genes upon chronic infection with *T. rangeli*. Samples of the brain and fat body from starved 5th instar nymphs uninfected and infected with *T. rangeli* CHOACHI strain were collected. Total RNA was extracted and sequenced in an Illumina HiSeq 2000. Bioinformatic analysis was used to identify and characterize the transcripts expressed in either tissue between infected or uninfected conditions. Data of the differential gene expression concerning the infection of *T. rangeli* was evaluated in the brain and fat body separately. We found differential gene expression in both tissues, as in the brain a total of 59 genes, and 38 genes in the fat body. According to the differential expression, 44 genes were upregulated and 15 downregulated in the brain. In the fat body, 21 genes were upregulated and 7 downregulated. Among the differentially expressed genes in the brain, there were those related to the endocrine control of molting (cuticle protein, ecdysis triggering hormone, and chitins), genes of enzymes important for digestion and nutrition (glycosidase, mannosidase and sugar transporter), and immune-related (lysozyme and phenol oxidase). Additionally, in the fat body, immune-related genes (defensins and lysozymes), and structural (cuticular protein) were upregulated. Phenotypically, insects infected with *T. rangeli* present trouble in molting and feeding. Those genes involved in these metabolic pathways might be triggering the effects.

Human versus computational algorithms in vector surveillance for cities

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Finding vector infestations in low prevalence settings is difficult but can be facilitated through rational use of historical vector information. However, health workers make decisions in the field based on perceptions and experience and may alter the potential efficacy of algorithms. In Arequipa, Peru, after a long spraying campaign for controlling Chagas disease, very few houses are infested with *Triatoma infestans.* Our aim was to compare alternative vector surveillance approaches: 1) Houses are assigned for inspection by a computer; 2) Inspectors are incentivized to choose high risk houses based on a modeling-generated risk map; 3) Current practice--inspectors choose houses with little or no prior information. These approaches were developed using the methodology accordingly: 1) based on a computerized algorithm, optimizing spatial coverage of higher risk houses, participants were told where to go, 2) using a behavioral economics approach to improve the participants' use of a risk map, and finally 3) the current practice using participants' experience. The nine participants entered data with a mobile app and searched 54 areas. We evaluated the main outcome using two methods that offered their own definition of surveillance success: 1) **"who won"**, based on number of won comparisons according the behavioral economics approach statistics, and defined by the relative importance of entomological surveillance objectives, and 2) **Difference and difference**, using the difference of mean probability of infestation given by a mathematical model before and after intervention. Computer algorithm beat current practice 13 of 16 times comparisons ($p=0.00854$), while behavioral economics beat current practice in 11 out of 15 ($p=0.0412$). We did not have enough power to compare the behavioral economics vs algorithm. Also, we developed mapped interviews that found participants were not comfortable working under the algorithm approach, since it takes out the capacity of decision from them. Algorithm and behavioral economics approaches achieved a good spatial coverage of higher risk houses, however, algorithm approach had bad impact into participants.

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Why all vector control programmes field technicians in Argentina are men?

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Chagas is a disease caused by the parasite *Trypanosoma cruzi* (T. cruzi) and a socio-environmental health problem that is present across the world. One of the routes of transmission of *T. cruzi* is through the contact with the feces of hematophagous insects, known as "vinchucas", infected with the parasite. In Argentina, since the 1960s, there have been operational programs which are responsible for preventing this way of transmission by eliminating the presence of these vectors through entomological surveillance and spraying homes and the surrounding areas with insecticides. Specifically, the people who carry out this task are the field technicians, who according to our observations, are all men. Thus, we can affirm that it is a totally masculinized type of employment. In this study, we tried to find answers about why women or other sex-gender identities do not participate in this occupational area. For this purpose, we interviewed thirteen field technicians, eight people in charge of the management and administration of the operational programs, and eight researchers who work on the Chagas issue in our country. Additionally, we interviewed two referents of the National Chagas Program and we carried out a documentary analysis of the current regulations on vector control. The majority of the people consulted expressed, following stereotyped gender criteria, that women do not have a profile compatible with this type of employment. Others indicated that there is a regulation that prohibits it, since insecticides could cause damage to women's fertility. A minority group indicated that it is a sexist system that, due to prejudice, does not allow women to be employed. Finally, we found that there is occupational segregation by gender towards women and other sex-gender identities, since there are no official recommendations or regulations which manifest that women should not perform this type of job. Likewise, we found that there were women (housewives, health and administrative agents) who performed the work of a field technician but without a formal contract, and also some of the people interviewed believe that it would be convenient to incorporate female field technicians. All in all, we consider that gender diversity is necessary in the work teams because it would improve interventions and strategies for vector control in the different territories. So, field technicians, both men and women, must have adequate training and appropriate personal protective equipment (among other strategies on occupational safety) to prevent contamination and intoxication with insecticides.

Effects of resistance and sublethal doses of deltamethrin on mating behavior and reproductive efficiency in *Triatoma infestans*

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Chagas disease is one of the most important human parasitic diseases in America and *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) is its main vector in the south of the continent. Chemical control of vector insects, mainly with pyrethroid insecticides, is the main way to reduce the incidence of the disease. However, the use of insecticides has limitations. On the one hand, resistance to pyrethroids has been detected in *T. infestans* in several areas of its geographical distribution. On the other hand, the insecticide applied in houses does not reach all individuals in the same way and/or is degraded by the environment, determining that some individuals are exposed to sublethal doses. Previous studies have shown that resistance to pyrethroids and sublethal doses of these insecticides can affect different biological processes of *T. infestans.* The objective of this study was to compare the reproductive efficiency and mating behavior between deltamethrin-resistant and susceptible *T. infestans*, exposed or not to sublethal doses of deltamethrin. Susceptible and resistant insects obtained from colonies that originated from insects collected in the Gran Chaco (Argentina) were used. Fifth instar nymphs (female and male) of both toxicological phenotypes were separated and exposed to a sublethal doses of deltamethrin every 15 days during the stadium. Treatments were applied: control (without exposure), exposure to acetone (Ac) and exposure to deltamethrin (D). Ten couples with the same treatment were observed for each experimental group and for each couple mating behavior variables (total mating time jumping onto, mounting, rejections, time elapsed until copulation, total mating time, post copulation time) and reproductive efficiency variables (numbers of copulas, time before oviposition, fecundity, fertility) were registered. Results showed that sublethal exposure did not affect mating behavior, but pyrethroid resistance increased male mounting number and female rejection tendency, and decreased total mating time. Regarding reproductive efficiency, the resistant insects showed higher numbers of copulas and lower fertility compared to susceptible ones. Females exposed to deltamethrin showed lower number of copulas, fertility and fecundity, and longer pre-oviposition time than control females. Thus, these results suggest that the toxicological phenotype and sublethal exposure to deltamethrin negatively affect the reproductive performance in *T. infestans.*

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Baited traps for early detection of triatomines in dwellings of indigenous communities of the Paraguayan Chaco region

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In order to evaluate the capacity of attraction of different aliphatic aldehydes to intradomiciliary triatomines, a field trial was conducted in four indigenous communities of the Paraguayan Chaco, all of them endemic for Chagas disease: 12 de Junio, El Martillo, Karandillas and Tiberia. After one month of complete spraying and being declared negative for triatomines at the beginning of the study, two traps were nailed on opposite walls of each inhabited house, 1.5 m above ground level; one was baited with an attractant and the other was an unbaited as control. Traps were not placed in peridomestic structures. A total of 139 baited traps and their controls were observed and checked after 1, 3 and 6 months of exposure. Four different triatomine attractants were tested: hexanal, nonanal, benzaldehyde, and heptanal at 98% purity or mixtures of them in different proportions. Attractants were placed in a hydroxyapatite tablet at a dose of 250 mL per trap. Statistical analysis was performed using Fisher Exact Test odds ratios (ORs) and p-value < 0.05, for all independent observations and McNemar's test p-value with continuity correction for each attractant. During the study, a total of 1,696 observations in baited traps and their controls were undertaken and 39 triatomines were captured inside the traps, corresponding to the species *Triatoma infestans, Triatoma platensis, Triatoma sordida,* and *Triatoma guasayana*. Overall, our results suggest a better performance of baited than control traps. (Fisher OR 2.90 CI 1.22 ± 6.89; $p< 0.02$) The capacity of attraction with the nonanal + hexanal mixture showed a highly probability of finding triatomines in traps with the mixture than without it (McNemar $p< 0.04$), enhancing the capture of triatomines in the traps in the first month of exposure. The probability of triatomine capture in traps with attractants in dwellings that were infested before spraying is 3.45%. *T. infestans* is no longer the predominant species in these communities with a history of high infestation of this species. Indeed, currently, adult *T. sordida* captures were predominating throughout the study in the traps as well as in the peridomicile by 1 man*hour manual search. The early detection of reinfestation or repopulation of intradomiciliary triatomines in the post-spraying period highlights the importance of this instrument as a useful tool for the early detection of triatomines in endemic areas and a support for communities under entomological surveillance.

Characterization of the Major Facilitator Superfamily (MFS) proteins of *Rhodnius prolixus* and study of their role in detoxification

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Chagas disease affects around eight million people worldwide. The causative agent is the protozoan parasite *Trypanosoma cruzi*, transmitted to mammals by the feces of triatomine insects. The most important vector species are *Triatoma infestans* in the Southern Cone and *Rhodnius prolixus* in the Andean region and in Central America. Given the absence of vaccines and efficient treatments in the chronic stage, reduction of triatomine population is the method choice for the control of disease. Vector control efforts have been based mainly on the application of pyrethroid insecticides. However, in the last decades it has been repeatedly reported the presence of *Triatoma infestans* after spraying with pyrethroid insecticides, which was associated with the emergence of insecticide resistance. Hence, the study of detoxification mechanisms and the discovery of targets for new insecticides are fundamental research fields for the improvement of pest management strategies. The MFS proteins are ATP-independent and transport a wide range of small solutes in favor of an electrochemical gradient. Previous results of our group indicated a decrease in the expression of some MFS genes after treatment with deltamethrin in *T. infestans* and after intoxication with an essential oil in Aedes aegypti larvae. These results suggest that some MFS transporters could have a role in the response to xenobiotics. In the present work we carried out a study of MFS proteins and their implication in detoxification in triatomines using *R. prolixus*. With the objective of performing a complete characterization of MFS transporters in *R. prolixus* physiology and their role in the response to a toxic compound, we combined bioinformatic and molecular strategies. First, we performed a phylogenetic analysis of these proteins along with those previously reported in *Drosophila melanogaster and T. infestans*. These results allowed us to identify possible candidates to be involved in the response to toxic compounds according to previous results found in those species. We found 7 candidates (RPRC010040, RPRC002229, RPRC010038, RPRC010027, RPRC008199, RPRC003332 and RPRC013855) and Selected RPRC010038 to analyze his expression in different tissues by RT-PCR. We found that it is expressed in crop, fat body, salivary glands, Malpighian tubules, nervous system and midgut, but not in rectum. Moreover, we performed quantitative PCR assays to evaluate gene expression changes after three hours of intoxication with the pyrethroid deltamethrin and we did not find significant changes from control. Here we present the first approach to the study of the role of MFS transporters in triatomine detoxification. The results contribute to the knowledge about detoxifying mechanisms in triatomine insects, valuable for the development of new vector control strategies.

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Chagas disease vector control: use of GIS and new technologies for surveillance and control in Cordoba, Argentina

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Spatial mapping tools are increasingly being used for disease surveillance, vector control, and health promotion activities. In particular, geographic information systems (GIS) have been introduced by different Chagas disease vector control programs. The use of these tools allows to analyze the distribution of vector species, develop transmission risk maps and outline different management strategies. In addition, the use of mobile devices allows faster information loading promoting more efficient data analysis. The Chagas Program (Cordoba Ministry of Health) proposes to interrupt the vector transmission of Chagas disease by eliminating the populations of *Triatoma infestans* present in houses, located within the endemic area. Since the middle of 2021, the Chagas Program has incorporated the use of mobile devices with GPS technology. Furthermore, a specific mobile app was developed (in EpiInfo^{TM} statistical software) to make possible georeferencing data and load other relevant information from each of the assessed houses. The main objective of this work is to illustrate the potential impact of the new implemented surveillance system through a description of the new methodology and the analysis of preliminary results obtained. By using this new surveillance system, it was possible to georeference a total of 10,510 houses corresponding to 9 of the 11 endemic departments of the Córdoba province. These values represent 42% of the total number of households estimated in the work area. It was also possible to calculate the infestation rates according to the presence-absence data of the vector species. Values of 0.65% and 7.5% were obtained for intra- and peridomestic infestation rate, with respect to the total number of houses analyzed. When the infestation rates were analyzed by each geographical state, the intradomiciliary rates showed a maximum value of 4.1% in Minas department (3 of 74 evaluated houses with *T. infestans* present within the dwelling). Moreover, the peridomestic infestation rate showed a maximum value of 14.4% in Pocho department. Furthermore, a transmission risk map of the endemic area was developed, based on the georeference data and the information of the infestation rates. It was possible to detect specific departments and cities with a higher risk of vector transmission. This new implemented surveillance system make it possible a real time data entry, allowing a more efficient processing of the collected field information and perform a graphical analysis by the development of specific risk maps. These implemented techniques represent an essential tool for vector surveillance and the control of Chagas disease in the province of Cordoba.

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MOSQUITO-BORNE DISEASES (DENGUE, MALARIA, YELLOW FEVER, ZIKA, CHIKUNGUNYA)

Effect of detritus decomposition time on *Aedes aegypti* oviposition site selection and immature developmental success

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The abundance of the mosquito *Aedes aegypti* in larval habitats (containers with water) depends on the selection of oviposition sites by the females and on the subsequent survival of the immatures. Both can be affected by factors such as the decomposition time of the detritus, which determines the number of microorganisms (larval food) in the habitat. Females would be expected to select sites where larvae have the best performance to maximize their fitness. The aim of this work is to study the relationship between the selection of oviposition sites in response to detritus age, and the survival of immatures in these sites. To achieve this, an oviposition study and an immature development study were performed. In both studies, three treatments with different decomposition times of detritus in water (3, 15, and 45 days) were used. The oviposition study was carried out in different locations (n=18) within Buenos Aires city. In each location, three ovitraps (black plastic containers with a detritus infusion) were exposed simultaneously, one for each treatment. After one week, the eggs per ovitrap were counted and compared between treatments. In the development study, larvae were reared in 200 ml plastic containers with 20 larvae each, in the same three treatments (n=6). Development time was recorded for each individual, and survival per container was calculated. Development time and survival were compared among treatments. The results showed a higher oviposition in the 14 and 42-days treatments (mean: 65 and 62 eggs respectively), as compared to the 3-days treatment (mean: 16 eggs). Larval survival was high in all three treatments (>90%). An effect of detritus decomposition time on the development time was observed, with shorter development times in the 3-days treatment (mean: 10 days), intermediate development times in the 14-days treatment (mean: 11 days), and longer development times in the 42-days treatment (mean: 13 days). Also a higher variability in the development times of individuals of the same treatment was observed for increasing decomposition times. The higher oviposition in sites with long decomposition times could be due to the accumulation of more microorganisms, which produce attractive chemical signals for females. The better performance of the larvae (represented by their shorter development time) in containers with shorter decomposition time could be associated with higher rates of reproduction of the microorganisms that constitute the larval food. These hypotheses, which should be addressed in future studies, might explain the lack of consistency between oviposition site selection and larval performance of *Ae. aegypti* observed in our study.

Assessment of arboviruses circulation in wild rodents and birds in the Upper Paraná Atlantic Forest

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Arboviruses are transmitted by hematophagous arthropods, many of them of sanitary importance for their impact on health, economy and their wide geographic distribution. In the sylvatic cycle, different wild vectors and hosts are involved. Some of these viruses belong to the Flavivirus and Alphavirus genera and are transmitted by different species of mosquitoes. Several wild vertebrates participate in the sylvatic cycles of these viruses as main or alternative hosts. Our aim was to evaluate the circulation of Flavivirus and Alphavirus genera in the Upper Paraná Atlantic Forest (UPAF) of Misiones, Argentina. We conducted a serological survey in April 2021 in three protected areas: Piñalito, Cruce Caballero and Moconá Provincial Parks, where we captured small rodents with Sherman-like traps, and birds with mist nets. We identified captured individuals at level specie and collected serum samples according to recommended veterinary protocols. We tested antibodies for Flavivirus: Yellow fever virus (YFV), West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), Ilheus virus (ILHV), and Alphavirus: Madariaga (MADV), using the plaque reduction neutralization test under agarose. We captured a total of 112 rodents (Piñalito: N = 52, Cruce Caballero: N = 28 and Moconá: N = 32) and 109 birds (Piñalito: N = 55 and Moconá: N = 54). We analyzed the overall prevalences of SLEV, MADV, and YFV for rodents and SLEV, WNV, MADV and ILHV for birds. In rodents, the overall seroprevalence of arboviruses was 1.92% in Piñalito (N = 52 MADV in Akodon montensis), 3.57% in Cruce Caballero (N = 28, MADV and YFV in A. montesis) and 0% in Moconá. In birds the overall prevalence of arboviruses was 1.81% in Piñalito (N = 55, SLEV in Conopophaga lineata) and 1.85% (N = 54, WNV in Cyanoloxia glaucocaerulea) and 1.96% (N = 51 ILHV in Leptotila verreauxi) in Moconá. These results represent novel information about arbovirus circulation in rodents and birds in the UPAF. Our results highlight circulation of different arboviruses in the UPAF, whose main or alternative hosts have yet to be identified. The study of these hosts could yield novel information about the eco-epidemiology of arboviral diseases of importance to human and animal health, and provides novel tools to use in the implementation of public health programs.

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New record of *Haemagogus janthinomys* (Diptera:Culicidae) in Chaco Province, Argentina, main vector of *Yellow fever virus*

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Yellow fever is an endemic zoonotic disease from Africa and America, with an urban and a sylvatic cycle. Mosquitoes are vectors of *Yellow fever virus* (YFV) in both cycles, but different species are involved in each one. In America, species belong to the genera *Haemagogus* and *Sabethes* are considered as main and secondary vectors in the sylvatic transmission, with *Haemagogus janthinomys* as the main vector species. During the last years, neighboring countries of Argentina: Brazil, Bolivia and Paraguay, reported YF outbreaks each time closer to the border. Regarding geographical distribution, this species is distributed from northern Argentina and southern Brazil to Honduras and Nicaragua. In our country *Hg. janthinomys,* inhabits the provinces of Salta, Jujuy, La Rioja, Tucumán and Catamarca so far. The objective of this work is to update the distribution patterns, mainly in the north of Argentina where this species has not been recorded. The sampling was made in "El Impenetrable" National Park, Chaco province, during february 2022. Adult mosquitoes were captured with entomologic nets and CDC traps (supplemented with CO2) and immature stages were collected from tree holes using pipettes. Some larvae were reared until adult emergence while others were stored in 70 % alcohol for future studies. We found a total of 17 specimens morphologically similar to *Hg. janthinomys* species (15 females and 2 males). Regarding sampling methods, 65% (n=11) was captured with entomologic nets; 6% (n=1) with CDC traps; and 29% (n=5) (immature stages) with pipettes. Due to the difficulty of distinguishing females of *Hg. janthinomys* and *Hg. capricornii* species, because of their morphological similarity, we proceeded to amplify a segment of the mitochondrial gene COI (cytochrome oxidase c subunit I) for molecular identification of species. In order to obtain the COI sequences, one leg from each of three specimens was removed and standard protocols to DNA purification and sequence amplification were performed. The COI sequences performed by Macrogen were compared with those stored in the BOLD repository. We found 94% of similarity with previously published sequences of *Hg. janthinomys*. This study is the first record of *Hg. janthinomys* in Chaco province (and Chaco Seco ecorregion) expanding its distribution, and determines a starting point for ecological studies.

Searching for potential bridge vectors in the mosquito community of equestrian facilities

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Arboviral encephalitides are a growing global concern. In temperate Argentina, St. Louis Encephalitis and West Nile viruses have been isolated from birds and mosquitoes, along with sporadic human cases. Both diseases present similar transmission dynamics, with an enzootic cycle maintained among wild birds and epizootic / epidemic cycles which may affect horses and humans among a wide range of mammals. While the mosquitoes grouped in the *Culex pipiens* L. complex have been incriminated as cosmopolitan vectors of these diseases, the role of other coexisting species is unclear and local cycles are poorly understood. In this context, mosquito blood feeding patterns are a key component in the dynamics of disease transmission, as they represent the nexus between birds and mammals. For most of the mosquito species in Buenos Aires Province, there is no information on their feeding habits. Our objective was to characterize blood feeding sources of usually ignored mosquito species inhabiting equestrian facilities in temperate Argentina, to identify potential bridge vectors. Adult resting mosquitoes were collected monthly in urban and rural fields from December 2018 to April 2019 with a battery-powered aspirator. Engorged females were identified to species with taxonomic keys and the blood source for each specimen was identified using molecular techniques. Laboratory work included mithocondrial DNA extraction, PCR amplification of a conserved fragment of the COI gene, sequencing of the amplified fragment and comparison with available sequences in GenBank. A total of 73 blood meal sources of female mosquitoes from 8 species were identified: *Aedes albifasciatus* (4 specimens), *Ae. crinifer (*23), *Cx. apicinus* (14), *Cx. chidesteri* (2), *Cx. dolosus* (7), *Cx. eduardoi* (9), *Cx. lahillei* (1) and *Cx. maxi* (13). From them, 13 vertebrate species were recognized as hosts, five of them were mammals (cow *Bos taurus*, dog *Canis lupus*, horse *Equus caballus*, brown hare *Lepus europaeus*, sheep *Ovis aries*) and the remaining were birds (chalk-browed mockingbird *Mimus saturninus*, monk parakeet *Myiopsitta monachus*, house sparrow Passer domesticus, spot-winged pigeon *Patagioenas maculosa*, picazuro pigeon *Patagioenas picazuro*, whistling heron *Syrigma sibilatrix*, house wren *Troglodytes aedon*, rufous-bellied thrush *Turdus rufiventris*). Four mosquito species (*Cx. apicinus, Cx. chidesteri, Cx. dolosus and Cx. eduardoi*) used both avian and mammalian hosts, and presented at least one feed on horse. Therefore, these species represent a potential risk of pathogen transmission from avian enzootic cycles to horse epizootics.

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Diversity of *Aedes aegypti* breeding sites in a neighborhood of the Metropolitan Area of Buenos Aires

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The *Aedes aegypti* mosquito is associated with the domestic environment, since it uses artificial containers with water located inside houses as breeding sites. This is an important species for public health because it is the main vector of several viruses such as dengue. In 2020, in was registered the largest epidemic of dengue in Argentina with more than 12,400 confirmed cases in the Metropolitan Area of Buenos Aires (MABA). Although, houses constitute most of the urban space coverage, there are practically no studies exploring the diversity and importance of mosquito breeding sites in this environment. Several studies have emphasized the need for an intersectoral approach to dengue control that combines environmental management practices with community participation. The aim of this work was to study the diversity of containers that can function as larval habitats of *Ae. aegypti* in relation to different physical aspects and socioecological characteristics of the inhabitants of the Mariano Moreno neighborhood, in the town of Claypole, in MABA. A sampling of the containers with water was carried out in the houses during the months of February and March, working together with the neighborhood referents. For each container, the presence of *Ae. aegypti* larvae and/or pupae, type of container and material were recorded. In addition, in each house sampled, a survey was carried out to find out if its inhabitants identified the mosquito breeding sites, and knew the prevention and control measures. The results showed that 60 of the 100 houses visited had mosquito breeding sites. A total of 334 containers were analyzed, and *Ae. aegypti* larvae or pupae were found in 132 (39.5%). The most common water containers were buckets, representing (22.8%) of the total containers (76/334). The main breeding sites were tires (95%) and the most frequent were plastic containers (39.4%). Of the 34 people who were aware of not accumulating water containers or invert them as a preventive measure against dengue, (53%) had breeding sites in their houses. The main results show that a large proportion of the houses had breeding sites and, in general, the inhabitants of the houses were aware of the main preventive measures. In conclusion, the practices of preventive measures, although they are known by people, are not successfully incorporated into daily life. The challenge is develop proposals of environmental management practices to reduce breeding sites with community participation.

The larval midgut of *Anopheles*, *Aedes*, and *Toxorhynchites* mosquitoes (Diptera, Culicidae): a comparative approach in morpho-physiology and evolution

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The midgut of the larval stage of mosquitoes is responsible for storing most of the nutrients that will sustain the events of metamorphosis and the insect's adult life. Despite its importance, the basic biology of the organ in the larval stage is poorly understood. To help fill this research gap, we carried out a comparative morphophysiological investigation of three larval midgut regions (gastric caeca, anterior midgut, and posterior midgut) of phylogenetically distant mosquitoes: *Anopheles gambiae* (*Anopheles albimanus* was used alternatively in some techniques as an anopheline representant), Aedes aegypti, and *Toxorhynchites theobaldi.* Larvae of Toxorhynchites mosquitoes are predacious, in contrast to the other species, that are detritivorous. In this work, we show that mosquitoes share basic histological characteristics of the organ but differ in others. The histochemistry of the larval midgut of *An. gambiae* indicates the existence of a different lipid and carbohydrate metabolism compared with *Ae. aegypti and Tx. theobaldi*. The gastric caecum was the most variable part among species and revealed particularities that are probably related to the chemical composition of the diet. The peritrophic matrix was morphologically similar in the three species, and the processes involved in the post-embryonic development of the organ, such as cell differentiation and proliferation were also similar. FMRF-positive enteroendocrine cells appeared grouped in the posterior midgut of *Tx. theobaldi* and individualized in *An. gambiae and Ae. aegypti*. Based on our findings, we hypothesized that predation by *Tx. theobaldi* larvae is an ancestral condition in mosquito evolution.

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Oviposition activity of *Aedes aegypti* in a temperate city of Argentina and its implications on local transmission of dengue

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The global incidence of the disease caused by the dengue virus has significantly increased throughout the last decades, becoming an emerging public health issue. Buenos Aires City, located in the temperate region of Argentina, has been affected by dengue epidemics during 2009, 2016 and 2020, with increasing number of cases. The main vector of dengue and other diseases such as Zika, Chikungunya fever and yellow fever is the mosquito *Aedes aegypti*, and the transmission of dengue is expected to be positively related to the abundance of this vector. The aim of this study is to analyze how the incidence of dengue cases during the last epidemic of dengue is related to the abundance of *Aedes aegypti* in different administrative districts within Buenos Aires City. The oviposition activity was monitored with ovitraps (n=218) distributed across Buenos Aires city, in the context of the monitoring program performed by our research group through a covenant with the city’s government. The period analyzed in this study was the summer season 2020, from January 1st to March 20th (11 weeks), when the monitoring was interrupted because of the Covid-19 pandemic. The data of dengue cases for the 2020 period were obtained from the Weekly Epidemiological Bulletin published by the government of Buenos Aires City. The oviposition activity was calculated as the proportion of positive records (i.e. number of times a trap had eggs divided by the total number of times the trap was active). Cumulative oviposition activity was calculated for each administrative district (Comuna) as the mean activity of all ovitraps in the Comuna. Three epidemiological variables were considered for each Comuna: the autochtonous/imported case relationship, the total number of cases, and the number of cases per 100000 inhabitants or prevalence. The relationship of these variables with the oviposition activity was analyzed. Oviposition activity varied among Comunas (20.7-80), as well as the autochtonous/imported case relationship (2.7-49.8), the total number of cases (23-1459), and the prevalence (16.4-641.6). A positive relationship of the three epidemiological variables with oviposition activity was observed. Epidemiological variables increased drastically at oviposition activity values over 60%. We discuss the effect of social-environmental heterogeneities within Comunas, and the spatial distribution of entomological and epidemiological variables. According to the results, we emphasize the importance of focusing prevention in areas with the highest oviposition activity, together with deepening control measures, which may reduce oviposition activity to levels below the threshold that allows for high transmission rates.

Diversity of mosquitoes (Diptera:Culicidae) and detection of arbovirus in a fragmented landscape of Morelos, Mexico

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Mosquitoes are among the most important vector species; they are responsible for 830,000 deaths per year owing their ability to act as vector of several pathogens. Around 100 of the 3500 species recognized as competent vectors, however, the role as competent vectors is unclear for a lot of species. Fragmented landscape where wildlife, farmlands and rural areas coexist, make up a large part of Mexico landscape. These areas are where spillbacks can take place, arbovirus can be stablished among local fauna and act as source of new outbreaks. It is necessary to understand the role that local species of mosquitoes play in the arbovirus cycle to support better public health decisions. This work aims to explore the diversity of mosquitoes in Sierra Monte Negro, Morelos, and the arbovirus among mosquitoes species. During July and August 2021, using CDC traps, mosquitoes were collected through two transects going through farmland, protected natural areas and rural landscape. Species were identified through morphological characteristics, pools were made of females of the same place, same species, and same month; a maximum of 15 individuals were put in these pools and were macerated in maintenance medium. Total RNA was extracted, and arbovirus were detected using quantitative reverse transcription PCR (qRT-PCR). A total of 22 mosquito species were found in the area, four of them are new records for the state, *Aedes podographicus*, *Ae. vexans*, *Culex sandrae*, and *Cx. interrogator*. The abundance of the species changes among farmland, protected areas and rural landscape; *Cx quinquefasciatus*, was the most abundant species of all the study, being present in all landscapes as well as *Ae albopictus*. *Haemagogus* species where present only in protected areas, on the contrary, *Ae aegypti* was mainly found in rural landscape. Zika and dengue virus were detected in pools of mosquitoes, most positive pools were from rural areas, followed by transition areas (points were protected areas end and some human activity is taking place). *Ae aegypti* and *Ae albopictus* pools were the main positive pools in the rural areas, however, in transition and protected areas we found pools from other species to be positive. Important information is missing since we don’t know if *Ae trivittatus* and *Ae podographicus* are competent vectors for zika or dengue virus, however, pools from these two species are positive for these arboviruses. This study highlights the importance of extending entomovirological surveillance to more species than *Aedes aegypti* and *albopictus,* as well as understand the vector competence of other mosquito species. Disease and vector ecology are fundamental in public health measurements, without these two disciplines the problem that public health face with arbovirus cannot be properly address, a multidisciplinary approach in necessary for arbovirus control in Mexico.

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Determination of antibodies to Flavivirus in wild birds from Buenos Aires City

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Saint Louis encephalitis virus (SLEV), West Nile virus (WNV) and Ilheus virus (ILHV) are flaviviruses maintained in nature by enzootic transmission networks between mosquitoes and birds. These viruses have been detected in South America and identified as a cause of neurological diseases in humans. There is a record of activity in monkeys and birds for ILHV in Chaco, Corrientes and Misiones; of WNV in horses and birds in the center and east of the country, while SLEV is widely distributed in Argentina and there is virological and serological evidence in birds, humans and various mammals. In order to detect viral circulation in free-living birds in the Autonomous City of Buenos Aires (CABA), seasonal sampling was carried out in the Costanera Sur Ecological Reserve. The birds were captured by means of mist nets, 0.1-0.2 ml of blood was collected from the jugular or brachial vein of 100 birds during 2016-2017. The serum obtained was analyzed for the detection of neutralizing antibodies using the technique of 80% plaque-reduction neutralization tests under agarose in monolayers of VERO cells. The highest seroprevalence was found for ILHV, which was 7% (n= 7/100), followed by SLEV whose prevalence was 4% (n= 4/100), and no positives for WNV. All reactions were verified as monotypic. The positive samples for ILHV correspond to *Zenaida auriculata* (n=3), *Saltator aurantiirostris* (n=1), *Leptotila verreauxi* (n=1), *Turdus amaurochalinus* (n=1) and *Turdus rufiventris* (n=1); for SLEV corresponded to *Turdus rufiventris* (n=2), *Mimus saturninus* (n=1) y *Turdus amaurochalinus* (n=1). These findings constitute the first evidence of circulation of ILHV in birds from CABA, considering that antibodies to SLEV were detected in *Zenaida auriculata* species, in 2015 with a prevalence of 7.2%. WNV has been detected in nearby towns, but not in CABA. In the future, it would be interesting to inquire broader ecological studies to deepen our knowledge of these pathogens present in our region.

Diversity, abundance, and presence of mosquito vectors of yellow fever in Northeast of Corrientes province

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Yellow fever is currently increasing in importance in Argentina due to outbreaks in neighboring countries such as Brazil and Paraguay that cover a great part of the Misiones and Corrientes border. Due to several epizootic events in the south of Brazil between 2020 and 2021, in March and April 2021, we perform a mosquito survey to evaluate abundance and distributions of species vectors of *Yellow fever virus* (YFV) in several selvatic locations with presence of monkey *Alouatta caraya* in Northeast of Corrientes province: Las Marias, Tají Poty, Garabi, Garruchos, San Carlos, Colonia Liebig and near the Chimiray stream. Adult mosquitoes were collected using nets and manual aspirators and were grouped per hour between 10:00 h and 15:00 h. Female adults were identified using general dichotomous keys. Relative abundance of each species and Shannon index of diversity (H) per site were calculated. For the most abundant species, the number of individuals captured per person (ind/per) was evaluated based on the time of capture through GLMM. We collected 676 mosquitoes belonging to 8 genera and 18 species. The most abundant species were *Aedes scapularis* (33.58%), *Sabethes albiprivus* (20.27%), *Ae. albopictus* (17.75%), *Haemagogus leucocelaenus* (15.86%), y *Psorophora ferox* (5.32%). *Aedes scapularis* was dominant in Garabi, Las Marías and Chirimay; *Hg. leucocelaenus* in San Carlos and Colonia Liebig; *Sa. albiprivus* in Taji Poti; and *Ae. albopictus* in Garruchos. The highest diversity was found in Garabi (H = 1.74) while the lowest is represented by Las Marias (H = 0.56). Only *Sa. albiprivus* and *Ae. albopictus* showed significant differences in the number of individuals captured between hours. The first presented a high value of 0.78±0.30 ind/per between 13-14hs; and the second a maximum value of 0.10±0.10 ind/per between 10-11 hs. On the other hand, *Ae. scapularis* presented a capture rate per hour of 0.69±0.39 ind/per, *Hg. leucoceleanus* 0.21±0.15 ind/per, and *Ps. ferox* 0.11±0.11 ind/per. The present work shows that the most abundant species in sylvatic environments of the northeast of Corrientes are vectors of YFV. *Sabethes albiprivus* presents a maximum of activity at midday, *Ae. albopictus* in the morning, and the rest of the species with constant values of activity between 10 and 15h. Enhanced studies about presence, diversity and abundance of mosquito vectors are necessary to identify epidemiological risk areas in order to implement vaccinations campaigns.

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Possible biotic interactions that are affecting the presence of *Aedes albopictus* (Diptera: Culicidae) in Misiones, Argentina

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In Argentina, despite local *Aedes albopictus* presents a certain degree of egg dormancy that would allow to be present at temperate latitudes its distribution is limited to two provinces with a subtropical climate: Misiones and Corrientes. Therefore, it is possible that other factors are limiting the expansion of this vector. To evaluate possible interactions that are affecting population and spread of this specie, we sampled artificial breeding site in Eldorado (urban environment) and Colonia Aurora (rural environment), Misiones province, in October 2015, April and November 2016 and April 2017. We performed generalized linear mixed models to evaluate which variables (type of container, volume of water, and environment) are associated with the presence of *Ae. albopictus, Ae. aegypti*, and mosquito predators (*Lutzia bigoti* and *Toxorhynchites* spp.). Also, in each environment, relationship between *Ae. albopictus* and *Ae. aegypti* was quantified through Hurlbert's C8 association coefficient. In Eldorado we collected 17195 larvae from 258 containers. *Aedes aegypti* was dominant (66.69%), while *Culex quinquefasciatus* represented 19.06% and *Ae. albopictus* 6.49%. In Colonia Aurora, of 5319 specimens collected from 149 artificial breeding sites, *Cx. quinquefasciatus* was the most abundant species (38.50%), *Ae. albopictus* was the second most abundant (23.82%) and *Ae. aegypti* accounted 12.24%. The presence of *Ae. albopictus* was higher in the rural environment (probability: 0.25±0.08) than in the urban environment (0.14±0.06) (χ2= 4.933; df=1; p= 0.026). It was also higher in containers with 1-10 liters (0.39±0.09) and 10-100l of water (0.42l±0.09) than breeding sites with 0-1l (0.05±0.04) and >100l (0.10±0.06) (χ2= 19.228; df=3, p=2; 453e-4). The presence of *Ae. aegypti* was associated with the environmnet (χ2= 36.196; df=1; p=1.784e-09). It was higher in the urban environment (0.96±0.02) than in the rural (0.38±0.08). The presence of predators was highest in the rural environment (χ2= 4.048; df=1; p=0.044), reaching 29.03% of the breeding sites in November 2016. Regarding C8 index, *Ae. aegypti* was negatively associated with *Ae. albopictus* in Eldorado city (C8=-0.105) and in the rural municipality, these species presented a positive relationship (C8=0.376). These results show that in the urban environments the high abundance of *Ae. aegypti* could be affecting the presence and abundance of the Asian tiger mosquito as evidenced by local studies of larval competition. And, in the rural environment, although the abundance of *Ae. albopictus* is higher, its presence is lower than the presence of *Ae. aegypti*. Possibly, a higher presence of predators in the rural environment is also conditioning the Asian tiger mosquito.

Host-feeding preference populations of culicidae domiciliary metropolitan area of Monterrey in Nuevo León

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Knowing the feeding patterns of mosquitoes helps us understand pathogen transmission cycles and determine the role of the vector in the maintenance and epidemic transmission of arboviruses, which leads to the development of more efficient measures to control diseases and vectors. In order to determine the blood feeding preference of Culicidae collected from two locations within the Monterrey metropolitan area, PCRs were performed on DNA extracted from feeding mosquitoes using vertebrate-specific cytochrome b primers. The results of the analyzes indicated that 19% of the *Ae. aegypti* fed on human blood, 35% dogs, 28% birds and 19% cats, does not agree with previous studies carried out, where it was found that *Ae. aegypti* fed mainly on humans, however, a similar study conducted recently in the United States and Mexico in southern Texas reported a similar result to ours, where 70% of *Ae. aegypti* fed on non-human hosts, with dogs being its main source of food. While 37% of the *Ae. albopictus* fed on humans, followed by 33% birds, 25% dogs, and 6% cats. This differs from what was previously reported in which it is considered an opportunistic feeder species with a predilection for feeding on mammalian hosts, however, we can find several studies where this species usually feeds on the blood of birds collected in an area where there is a great availability. Regarding the species *Cx. quinquefasciatus* birds were its main host with 54%, 25% of blood intakes were from dogs, while in humans and cats 11%. Showing the expected behavior due to the fact that the main host of these were birds with more than half of the blood intakes, agreeing with previous studies where it was reported that most of the intakes of this species belong to birds. The percentages of multiple feedings that the mosquitoes of each species presented showed that 31% of the females of *Ae. aegypti* fed on more than one host, *Ae. albopictus* 35% of the fed mosquitoes fed on a single host and *Cx. quinquefasciatus*, 62% of the mosquitoes analyzed showed feeding on more than one host. These multiple feedings can increase the risk of disease transmission by increasing the frequency of contact with hosts.

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Aedes aegypti (Diptera: Culicidae) frequency in the state of Sergipe, Northeast Brazil

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Aedes aegypti (Linnaeus, 1762) is the main vector of dengue, chikungunya and Zika arboviruses, which cause important epidemics, mainly in the American continent. This mosquito is anthropophilic and very frequent in urban areas. In Brazil, the lack of basic sanitation in several areas, which involves irregular garbage collection and inadequate water supply, as well as the disorderly growth of cities, results in the provision of artificial breeding sites, contributing to the mosquito proliferation. The surveillance and control of *Ae. aegypti* are essential to mitigate the number of cases of arboviruses as Dengue, Zika and Chikungunya. The present study aimed to investigate the distribution and level of infestation of the state of Sergipe, Brazil by *Ae. aegypti.* From March 21 to April 25, 2022, entomological surveillance activities were carried out using ovitraps to monitor the presence of *Ae. aegypti* in eight municipalities: Umbaúba, Canindé de São Francisco, Pinhão, Capela, Laranjeiras, Simão Dias, Siriri and Neópolis. The number of ovitraps ranged from 50 to 100 per municipality, totaling 595 traps installed in different regions of the state. Ovitraps were assembled with one paddle and yeast as attractant. *Paddles* were collected weekly and replaced with new ones for two weeks. All *paddles collected* were sent to the Laboratory of Entomology and Tropical Parasitology for egg counting and hatching for species identification. Ovitrap positivity index (OPI = positive ovitraps/installed ovitraps*100) and egg density index (EDI = total eggs/installed ovitraps) were calculated. A total of 1,086 *paddles* were collected during the two weeks, 555 in the first week and 531 in the second week, accounting for a total of 96,604 eggs. The average positivity was above 60% for all cities, considering the two weeks of surveillance. Overall, positivity increased from the first to the second week. The cities with the highest infestation rate were: Siriri (84.0%), Capela (76.9%) and Pinhão (76.8%). Egg density index ranged from 51 eggs/paddle (Neópolis) to 208 eggs/paddle (Laranjeiras). All municipalities showed high levels of infestation by *Ae. aegypti* and these results were incompatible with official indices provided by the health services. Thus, it is necessary to carry out technical training activities, review methodologies, processes and information flows related to supervision in these municipalities, in order to investigate and correct the inconsistencies observed, in addition to the search for better sanitation conditions in these municipalities.

Survey of Culicidae from irrigation channels and draining ditches in Salta city, Argentina

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Mosquito studies in Salta province have mainly focused in the yungas, the second region with the greatest diversity of mosquitoes in Argentina. However, the Culicidae fauna of the Capital Department (Salta), where the city of Salta is located, is unknown. Salta city is the largest of the province, with a population of 533300 inhabitants and is located within the border of yungas and chacoan phytogeographic regions. It has a temperate climate with dry winter and mild summer. A survey was carried out of irrigation channals and draining ditches across the city. Eleven sites were sampled in sppring (between September and November 2021) and summer (between February and March 2022). In each site and season, 10 water samples were collected with 400 ml dippers; mosquitoes were counted, and a subsample of early instar larvae were bred to the four instar to aid in taxomomic identification, which was based on morphological characters. Over 90% of larvae collected were *Culex quinquefasciatus*, in both sampling seasons. In spring and summer a very low number of *Aedes aegypti* were detected, an unusual finding since the species develops mainly in water-holding containers. Other species found include *Culex maxi, Cx. tatoi* and *Anopheles* spp. The finding of *Anopheles* merits further attention; although Argentina was declared malaria-free since 2019, it is important to know where the vectors and potential vectors are present, for a better delimitation of areas at risk of reintroducing the disease. These results highlight that in Salta city, water ways should be monitored regularly for vector control because they are larval habitat to species of epidemiological significance such as *Culex quinquefasciatus*.

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Oviposition site choice and subsequent offspring fitness of *Aedes aegypti* mosquito in short- and long-term detritus accumulation treatments

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Larval habitats of *Aedes aegypti* are artificial containers with water, where the larvae depend on the external contribution of plant detritus as a source of nutritional resources. Containers with longer outdoor exposure accumulate more detritus than containers with recent outdoor exposure or some sort of regular maintenance. According to optimal oviposition theory, females lay their eggs in habitats that maximize the fitness of their offspring, i.e., containers of high nutritional quality. The aim of this study was to evaluate the relationship between the selection of oviposition sites by Ae. aegypti females and the subsequent success of the larvae in habitats with different detritus accumulation times. For this, outdoor containers were used, in which detritus were added 2 weeks before starting (short-time treatment: S) and 2, 4, 6 and 8 weeks before starting (long-time treatment: L). Detritus was collected during two weeks at different sites to represent the natural variability (i.e., each replica accumulated different quantity and quality of detritus depending on its location), then weighed and added to the container. The experiment consisted of two parts: 1) the selection of oviposition sites by females was studied in S and L containers. Here, the number of eggs and the number of eggs per gram of organic detritus were compared between treatments. 2) Larvae were reared at low densities in S and L containers to evaluate success during development. Here, a composite index of performance (λ') was calculated for each container based on larval survival, time to adult emergence, and female size. The effect of treatments (S, L) and of the amount of organic detritus on λ' was analyzed. The number of eggs was significantly higher in the L treatment than in the S treatment (mean: 193 and 55 respectively). However, since the L containers collected on average 4 times more organic detritus than the S containers, the number of eggs per gram of organic detritus was similar in both treatments. The performance in the containers was positively affected by the amount of organic detritus collected. No significant effect of treatment was detected, although a slightly higher developmental success in S than in L was observed. The higher number of eggs in the L treatment suggests a preference of females for laying eggs in containers with longer detritus accumulation time. However, at the low and constant densities used in this experiment, no differences in performance were detected between treatments. At larval densities related to the number of eggs actually laid, a worse performance would be expected in the most selected treatment, what should be addressed in future studies. The results of this study suggest that female selection of oviposition site may contribute to larval crowding and play a role in population regulation.

Larvicidal activity of plants of the Brazilian flora as an alternative measure to control *Aedes aegypti*

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The control of arboviruses is a challenge for global public health. In Brazil, climatic and environmental conditions favor the circulation of the main vector, *Aedes aegypti* (Linnaeus, 1762). Vector control is an essential process to prevent or decrease the circulation of arboviruses, which can be carried out in two ways: eliminating adult mosquitoes and breeding sites larvae. The Brazilian flora is very rich in specimens with pharmaceutical potential, being an alternative source for the development of larvicides, repellents and other products that can be used to reduce human-vector contact. The present study evaluated the larvicidal activity of eight essential oils from plants of the national flora. Dose-response bioassays were performed to verify the lethal concentration of the oils. In each test, four cups with 10 larvae of the third larval instar were exposed for each concentration. To the solution preparation, the mixture of two solvents was tested: Tween 80 (10%) and Dimethylsulfoxide (DMSO) (20%). An initial screening was carried out with concentrations ranging from 10 to 500 ppm. Reading was performed after 24 hours. After the screening, four oils that showed low toxicity to the larvae were excluded: *Lippia alba*, *Eucalyptus grandiose*, *Ocimum americanum* and *Tetradenia riparia*. The other oils were retested through concentrations ranging from 20 ppm to 200 ppm, in triplicate on different days and, later, the lethal concentrations that kill 50% of the larvae (CL_{50}) were calculated. The most effective oil was *Cymbopogon citratus*,CL_{50} 83.73 ppm, followed by *Corymbia citriodora* oil, CL_{50} 81.50 ppm *Cymbopogon winterianus* CL_{50} 83.73 ppm and *Ocimum gratissimum* CL_{50} 79.13 ppm. The oils with the greatest toxicity to the larvae were those with citral, citronellal and eugenol among their major compounds. This study showed the effectiveness of the plants from the Brazilian flora and their potential for the development of new larvicides.to control *Aedes aegypti.*

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The impact of polarized light and physical-chemical water bodies parameters on the malaria mosquito oviposition site selection

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Current mosquito control involves in-door interventions such as insecticide spray and mosquito nets are commonly used. However, these are nocturnal strategies, while evidence suggests that 20 to 30% of daily bites occur during the daytime. Larval source management (LSM) are promising new strategies. However, the *Anopheles* oviposition pathway remains unclear. Although the literature suggests the idea of *Anopheles* detecting potential breeding sites through light polarization and water quality based on its parameters. Since breeding sites mostly consist of a substrate, vegetation and fauna, their composition affects water chemical-physical parameters (total dissolved solids, pH, conductivity, temperature, dissolved oxygen, nitrate, phosphate, alkalinity and hardness), therefore, affecting the breeding sites. Moreover, given the evidence of the visual system for selecting breeding sites in other mosquitos, it is important to find out whether the use of polarization or physical-chemical characteristics have an important role in the oviposition site selection. Therefore, this study aims to have a better understanding of the factors driving *Anopheles* oviposition behaviour and its environment through analysis of chemical-physical properties and light polarization in water surfaces. We use quantitative analysis methods for the measure of physical-chemical parameters of water samples *in situ*. This analysis use colourimetry kits, multi-polarimeter and titration. In the horizontal polarization stage, we use camera lens filter (CPL and UV) in a range of degrees (0º, 90º and 180º). Then, we use the software AlgoNet for analyzing the degree of polarization of water body sample images. We use regression linear models for statistical analysis on the association assessment in the function of larva density. PCA correlation matrix was used for the analysis of water parameters distribution. The PC1 (30%) retains most of the variation, while the PC2 (15%) had a few positive correlation values. Hardness (0.5345) and alkalinity (0.52763) had a prominent effect on PC1. Distribution of the data values within the study sites have similarities in two groups: Canete and Mala, both in the rainy season; and Cieneguilla and Canete, in the rainy season and the dry season respectively. Within the physical-chemical water parameters, conductivity had a statistically significant association with the number of larvae found in breeding sites (p-value=.023863). CPL light polarization analysis indicated pH (p-value=.039780) and number of larva (p-value= .003571) to be statistically significant predictor variables in the model. UV light polarization analysis did not show statistically significance, as expected from the literature. Overall, we cannot conclude that water chemical-physical parameters or light polarization are the only factors involved in the oviposition behaviour of *Anopheles sp.* However, it may influence the survival rate of larvae, therefore having an indirect impact on the pathway of mosquito oviposition. Further research in a controlled and uniform environment is needed to avoid external variables conflict.

Mosquito fauna from the Urutaú Natural Reserve in Misiones, Argentina, with discussion about their potential as vectors of important pathogens

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Urutaú Natural Reserve is a protected area located in the south of Misiones province, Argentina, near Posadas Capital. This location is characterized by warm and humid climate that promote great flora and fauna diversity. Regarding the mosquito fauna, from 194 species recorded for Misiones province, 66 species are actually registered for Candelaria and 88 for Capital Department. It is known that many of them are vectors of pathogens and parasites that cause human and animal diseases. Our goal was to know the mosquito community in order to consider their importance in pathogens and parasites transmission and evaluate the performance of different capturing techniques. The study was performed between September 2018 and May 2019 through four surveys. We sampled both, adults and immature stages using light traps (CDC and Fabric trap) at night and nets with aspirators (Net/Asp) during the day. Immature stages were collected from different water bodies under the ground, foliar axils and tree holes. A total of 2541 adults and 24 immature individuals were collected. Comparing the number of species and the number of individuals captured as adult with different methodologies, Net/Asp was the most effective in capturing diversity of species while CDC captured significantly higher number of individuals but with lower diversity ($P<0.05$). We were able to confirm the presence of 30 mosquito species belonging to nine genera. Among them, *Culex* (*Culex*) *lahillei* for the first time in Misiones province. Moreover *Aedes* (*Protomacleaya*) *terrens* and *Culex* (*Melanoconion*) *ribeirensis* are new citation for Capital Department. From 30 species identified, 15 of them are suspected to be implicated in cycles of pathogen´s transmission. Many *Culex* species are suspected to be involved in encephalitis transmission cycle in Argentina: Cx. *delpontei*, *Cx. maxi* and *Cx. coronator* (*Venezuelan equine encephalitis virus*) and *Culex quinquefasciatus* (*Saint Luis encephalitis virus*). Other species are related to *Yellow fever virus* (YFV) in Brasil (*Psorophora ferox*, *Psorophora albipes*, *Aedes serratus*, *Aedes hastatus* and *Aedes scapularis*) and *Sabethes albiprivus* that is the only mosquito from which the YFV has been isolated in Argentina before. In addition, *Ae. scapularis* and *Cx. quinquefasciatus* transmit the parasite *Dirofilaria immitis* to human and domestic animals. The importance of knowledge about vector mosquitoes and the pathogens they transmit lies in their potential circulation in the native fauna, being a latent focus of possible future epidemic outbreaks in humans and domestic/wild animals.

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Spatiotemporal dynamics of dengue emergence in the city of Reconquista (Santa Fe, Argentina) and its relationship with meteorological and social variables

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Dengue fever is an important zoonotic disease in inter-tropical areas worldwide. In the past decades, the dengue virus (DEN) has spread into sub-tropical and temperate regions, with greater incidence and increasingly frequent epidemic outbreaks. In Argentina, DEN is an epidemic disease initiated by imported cases from countries where viral circulation is permanent. Santa Fe is a province in central Argentina where dengue outbreaks have been recorded since 2009, and the city of Reconquista has been the most affected since then. The biological characteristics of both the vector and the virus depend on the climate and the environment, which can therefore impact the number of registered cases at a given place and time. The aim of this paper is to begin evaluating possible relationships between the dengue cases that occurred in 2020 during the major epidemic in Reconquista and climate and social variables. The analysis is made using time series of the number of cases per epidemiological week (EW) in the 2019–2020 season, as well as the anomalies in minimum, maximum, and mean temperatures and total precipitation. The anomalies were calculated as the difference between the values of each EW in the 2019–2020 season relative to the reference period 1998-2019. Moreover, a count was made of the number of days with extreme temperatures above the 90th percentile (P90) and extreme precipitation above the 95th percentile (P95) in the EW with recorded cases, as well as in the four preceding and following weeks (with respect to the reference period 1998–2019). Finally, the incidence by neighborhood was calculated and related to the coverage of the sewer and drinking water supply networks, with no relationship identified. Dengue incidence was low to medium in most neighborhoods (76%) and high and very high in the rest (24%). The dengue cases occurred during the weeks with positive anomalies in minimum, maximum, and mean temperatures. Strong positive precipitation anomalies were observed in the weeks when cases began to appear. During the EW analyzed, 19, 33, and 21 days with minimum, maximum, and mean extreme temperatures, respectively, were identified. There are no records of extreme precipitation in the period under study. This paper is the first-ever spatiotemporal analysis of dengue fever incidence in the city of Reconquista, and sets the foundations for outlining public policies and actions for dengue fever prevention at the local level. The need has been identified for standardized and long-lasting epidemiological, meteorological, and social databases.

Mosquitoes (Diptera: Culicidae) in the Zoo, Aracaju Sergipe, Brazil

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The Atlantic Forest is a tropical forest considered one of the most diverse biomes of the planet, ocurring in 17 Brazillian states. Throughout the years 2016-2018, epizootics and historical epidemics of sylvatic yellow fever occurred in areas of Atlantic Forest in Southeastern states of Brazil. In Sergipe, in the urban area of the capital Aracaju, there is an Environmental Protection Area (APA) of Atlantic Forest, which has tourist attractions such as the zoo and a cable car. This study aimed to investigate the mosquito fauna, potential vectors yellow fever, in the APA area. For this purpose, immature specimes were collected using ovitraps at two heights, 1 and 5 meters, installed in two areas: the area the circulation of people and the forest area. The traps were checked once a week, when the paddles and larvae and pupae eventually present in the water were collected. After dry, positive padlles were immersed in trays with table water and food for eggs hatching. Larvae were reared until the 4th instar or until adult emergence, and identified according to available dichotomous keys. In 18 weeks research, 6567 eggs were collected, most of them, 61.9%, them in traps installed in the open area and 38.1% in the forest area. Regarding to the height of installation of the traps, it was observed that 76.2% of the eggs were collected at a height of 1m while 23.8% were collected at a height of 5m. These findings show the preference of species for low heights and greater demand for oviposition in areas where people circulate. To date, richness of three species has been identified in the ovitraps, *Aedes albopictus*, *Aedes aegypti* and *Toxorhynchites theobaldi*, with *Aedes albopictus* being the most abundant. The results point to the risk of transmission of arboviruses in the APA area, in view of the abundance of vector species. Wild species of yellow fever of *Haemagagus* and *Sabethes* genus have not been recorded so far.

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An exploratory research of morphological patterns of *Aedes albopictus* (Diptera: Culicidae) in Misiones, Argentina

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Aedes albopictus (Diptera: Culicidae) is a vector of several arboviruses, distributed from Misiones to the northern of Corrientes province in Argentina. Its wide distribution around the world has been attributed to rapid reproduction and adaptive capacity to different environments. The objective of this work was to search morphological patterns *of Aedes albopictus* populations, using wing geometric morphometric analysis. Our study area was the city of Puerto Iguazu and Iguazu National Park, characterized in three environments: urban (UR), periurban (PU) and sylvatic (SY). Ovitraps were placed in these environments to obtain eggs. The larvae from hatched eggs were reared in the laboratory until the emergence of adults. A total of 179 female wings were extracted and photographed. Seventeen (17) landmarks (LM) were placed on the images and a Generalized Procrustes Analysis was applied. Univariate and multivariate analyses were performed on the shape and centroid size. Wing size of PU was larger than SY (p-value=0.0096). In contrast, those of UR had intermediate size values and more variability. Heterogeneity conditions and resources present in urban environments could influence the size of females bred there and their offspring. The metric disparity index (as an estimate of wing shape variance) did not show significant differences among environments, although an increased variability was observed from the sylvatic environment (SY=0.00092) to the urban environment (UR=0.00101). This pattern could be associated with an environment more disturbed by human activity. Scatter plot of Canonical variate analysis showed overlap among the shapes of different environments, however, the permutation test was significant between UR and PU (p=0.0019) and between SY and UR (p=0.0457). Cross-validated reclassification had overall accuracy of 64 % with a higher percentage for UR (68.4%). Therefore, UR shapes could be partially differentiated by their morphological distances, observed in the cluster analysis and less classification error, indicating a particular or more dissimilar form. Wing morphology has differences among environments, and the shape variables discriminate most individuals. Anthropic processes which affecting the urban environment, as well as environmental characteristics, could be associated with variability in shape and size, and operate selectively on morphs. While between sylvan and peri-urban environments, less differences were observed which could be related to common characteristics that also affect the shape. These possible morphological structural patterns of *Aedes albopictus* could be complemented in the future by genetic studies.

Digestion-related proteins in the malaria vector *Anopheles aquasalis*

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Knowledge at the molecular level of malaria vector mosquitoes is fundamental to the development of effective interventions measures to control the disease. The publication of the *Anopheles aquasalis* genome will allow identifying target genes to creat new vector control tools, such as digestion, immunity, and detoxification genes. This work aims to perform the *An. aquasalis* genome annotation, identifies genes related to digestion and evaluates the phylogenetic relationship with other anophelines. Initially, a tBLASTn was performed using anopheline proteins as a query against the *An. aquasalis* genome, with e-value 10^{-5}. Unique scaffolds were selected. RepeatMasker was used to perform the identification of the repetitive sequences. Structural annotation was initially done with MAKER. After two rounds, the MAKER output was used to do the training and final annotation with AUGUSTUS. Functional annotation was done following the pipeline of Pannzer2 and InterproScan. The digestion genes described in the reference proteome of *Anopheles gambiae* were used as a query for identifying sequences in *An. aquasalis* by homology using BLASTp. For evolutionary analysis, a phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates in MEGA-X. Structural annotation identified 12,446 protein-coding sequences in *An. aquasalis*, classified as complete by BUSCO in 92.4% of the sequences. Functional annotation results showed that 10,590 proteins have a defined function, with 53.4% of the proteins classified in the molecular function category, 22.9% in biological processes, and 15.2% in cellular components. A total of 241 genes related to *An. aquasalis* digestion was identified: 56 genes related to carbohydrate digestion; 51 for lipid digestion; and 134 genes for protein digestion. Phylogenetic relationships with other anophelines showed that *An. aquasalis* genes are most closely related to the neotropical mosquitoes *An. darlingi* and *An. albimanus*. Groups of orthologous genes were conserved in important families of all four species. Some of these conserved genes may assist in vector control studies: larvicidal toxin receptor genes, such as alphaamylase; target genes for transmission-blocking vaccines, such as aminopeptidase N1 and carboxypeptidase B; as well as characterization of blood-meal digestive enzymes that may negatively or positively affect Plasmodium development in the midgut, such as serine proteases trypsin and chymotrypsin. Indeed, these data can be used to develop of new malaria control strategies.

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Study of emerging Flaviviruses transmitted by mosquitoes (Diptera) in a border area in northern Chile

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Flaviviruses are mosquito-borne pathogens classified as Arboviruses, a group capable of emerging and spreading to new geographic regions. Several events initiated by human activities like deforestation, urbanization and agricultural intensification have caused habitat fragmentation and loss of biodiversity. Such situations, coupled with a sustained increase in human population and climate change, are essential determinants contributing to the emergence of the diseases caused by flaviviruses. To date, continental Chile is free of Flaviviruses since no native human cases have been reported, despite the vectors being present, the re-entry of more aggressive vectors such as *Aedes aegypti*, and the movement of people and pets from endemic areas. Hence, we used mosquitoes as sentinels of these pathogens for the first time in continental Chile using RT-qPCR detecting Flavivirus in mosquitoes collected from the extreme North of Chile. Between 2019 and 2020, 1,148 mosquitoes (larvae, pupae and adults) were collected at 50 points in three regions (Arica y Parinacota, Tarapacá and Antofagasta). RNA was isolated with a commercial kit and then reverse transcribed to ss-cDNA. Detection by RT-qPCR was performed with specific-primers for Flaviviridae. As an internal control, Culicidae Actin primers were used. RT-qPCR assays did not detect Flavivirus in any of the mosquitoes collected. These results could indicate that the geographical isolation of the country and the strict border sanitary control have kept northern Chile free of various pathogens, including Flaviviruses. However, further surveillance activities are strongly recommended using a more significant number of samples.

The role of JNK, Fos and Puc immunity genes of *Anopheles aquasalis* during *Plasmodium yoelii* infection

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Malaria is a disease caused by protozoa of the genus *Plasmodium*, parasites transmitted to humans through an infected anopheline mosquito bite. The activity of the mosquito's immune system is one factor that interferes with the efficiency of infection and transmission of the protozoan to vertebrate hosts. Despite its importance, little is known about the immunological responses of vectors from the New World (Americas) in the fight against the parasite. Based on the scarcity of knowledge about the biology and immunology of the American vectors, the present study aimed to evaluate the influence of the JNK, Fos, and Puc immunity genes, components of the *Jun N-Terminal Kinase* (JNK) immune signaling pathway of the vector American *Anopheles aquasalis* infected with *Plasmodium yoelii*. Through the RNA interference (RNAi) technique, the immunity genes JNK, Fos and Puc were silenced and evaluated the impact of the infection on the anopheline. In addition, the expression levels of antimicrobial peptides (AMPs) defensin-2 and gambicin were evaluated before and after infection during Fos and Puc silencing. The silencing of the JNK gene in *An. aquasalis* reduced both intensity and infection rate compared to the control. Fos gene silencing reduced infection intensity, increased gambicin expression, and did not alter defensin-2 expression. Puc gene silencing did not impact infection or AMPs expression. The results of this study differ from those observed for *An. gambiae*, the primary malaria vector in Africa; but they resemble those of *An. stephensi*, an Asian vector. The JNK immune pathway was not efficient in combating *P. yoelii* in *An. aquasalis*, and the parasite reduction observed after silencing both JNK and Fos indicates that it reflects the action of an alternative immune pathway. These results increase the information regarding the interaction between anopheline vectors and *Plasmodium* that cause malaria, in addition to generating knowledge about the immune responses of an important American vector to infection by the protozoan. Complementary studies on the functioning of the various immune pathways and their possible interrelationships are necessary and have great potential to substantiate the development of new strategies to control and block the transmission of *Plasmodium* to vertebrate hosts.

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Evaluation of the effect of apoptosis on vector competence of mosquitoes infected with Zika Virus

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Currently many arboviruses of epidemiologic importance circulate in Brazil, where Dengue Virus (DENV) is the most important. The Zika Virus (ZIKV) recently arrived in Brazil in 2015 where many cases have been reported, concerning the Brazilian authorities. The main vector of these arboviruses is the *Aedes aegypti* mosquito, and another potential vector is the *Aedes fluviatilis* (Silva et al., 2017). Both species are wildly spread throughout South America, especially in Brazil, bringing risks and exposing the population to the viruses. An antiviral response to infection from the vector is the cellular self-destruction capacity through apoptosis. Some authors have shown that the vector competence of a mosquito species to a virus can be affected by enhancing the number of cells in apoptosis after an infected blood meal. The main goal of this work was to evaluate the effect of apoptosis in the midgut of the different mosquito species when infected with ZIKV. Female mosquitos of *A. aegypti* and *A. fluviatilis* were separated into two groups, where the mosquitos fed on infected blood and non-infected blood (control group) using experimental membrane feeders, and the midguts were dissected 7 and 14 dpi to 1) analyze the infection level (PCR); 2) check the caspase activity; 3) perform the tunnel assay. As a result, the *A. fluviatilis* showed low infectivity, with a 10% infection rate, while *A. aegypti* showed high infectivity, with a 70% infection rate. In addition, when comparing infection between species, the *A. fluviatilis* mosquitoes exhibited high caspase activity and cells in apoptosis. In contrast, the *A. aegypti* revealed low caspase activity and cells in apoptosis. It demonstrates that apoptosis may influence the ZIKV replication. Also, the susceptibility of *A. fluviatilis* to infection is inversely proportional to apoptosis level and vector competence, indicating a low capacity to transmit ZIKV to the population. However, more experiments will be necessary the affirm that the vector competence of *A. fluviatilis* is directly related to apoptosis.

Cellular and molecular aspects of the interaction between dengue and Zika virus with the mosquito vector

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Arboviruses are neglected diseases transmitted by mosquito vectors. Despite receiving greater recognition in the last decade, the number of cases and deaths is still alarming. Dengue and Zika are viral diseases that are part of this group and have a high incidence in tropical countries. Mitigation of such diseases consists mainly of population control strategies of mosquito vectors. In Brazil, the main vector of dengue (DENV) andZika (ZIKV) viruses is *Aedes aegypti*. Despite efforts to reduce cases of both diseases, the circulation of DENV and ZIKV generates frequent outbreak episodes. For this reason,studies involving the virus-vector interaction are necessary and can potentially contribute to arbovirus control strategies. The present study evaluates the vector competence of *A. aegypti* populations from an endemic city (Belo Horizonte/MG) for thetransmission of DENV and ZIKV in 7 days after infection (dpi) – initial infection and 14dpi – late infection; and describes the kinetics of viral replication at the cellular level through the morphological analysis of C6/36 cells. Our results showed that the infectionpatterns of *A. aegypti* populations are modulated according to the flavivirus studied, with no similarity between vector competence and viral load. Regarding DENV infection, depending on the analyzed population, vector competence may decrease, increase or remain the same from 7 to 14 dpi. For ZIKV, it increases or remains the same. During, ZIKV tends to accumulate in the salivary glands of vectors. At the time, forDENV, the accumulation of viral particles does not have a pattern, varying according to the population studied. Although DENV and ZIKV belong to the same viral family and are structurally similar, we observed differences in their infection profiles in C6/36 lineage cells. In the later period of DENV infection, we observed an intensification of the cytopathic effects, with reduction of actin filaments, formation of cell groups, and destruction of the monolayer. For ZIKV infection, we noticed the presence of numerousmultinucleated cells. Thus, we found that the differences in the cytopathic effects vary according to the pathogen studied and the post-infection time.

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Evaluation of *Plasmodium vivax* from treated patients able to infect *Anopheles aquasalis* and *Anopheles darlingi*: Effect of different drugs treatments

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Plasmodium vivax is the leading causative agent of malaria in the Brazilian Amazon region. The WHO recommends for first-line therapy the combination of chloroquine/primaquine. Furthermore, new drugs such as tafenoquine (8-aminoquinoline) are reported as a radical cure for *P. vivax.* In this study, we investigate the effect and /or interference of drugs taken by patients with malaria vivax in transmitting the infection to the *An. aquasalis* and *An. darlingi*. Patients are recruited from the Fundação de Medicina Tropical Heitor Vieira Dourado (FMT-HDV), Manaus, Amazonas, Brazil. They received different supervised therapeutic protocols. We are collecting blood samples from these patients before (T0) and after treatment every 8 h (T1, T2, T3) for up to 4 days post-infection (d.p.i.). *Plasmodium vivax* in the blood is confirmed and quantified by thick smear. The mosquitoes used are obtained from colonies established in the Department of Entomology of the FMT-HDV. They are being infected by membrane glass-feeding devices using whole infected blood from the treated patients and kept in the insectary until they be dissected at 8 and 15 d.p.i. When evaluating mosquitoes' infection with infected patients' blood at different times (up to 30 h), the presence and development *of P. vivax* oocysts in the mosquito's intestine is observed. This study aims to determine how antimalarial treatments works after the drug ingestion by the patients, and the time *P. vivax* remains infective to the vectors contribuiting to maintaining active foci. Resuming, we are testing the capacity of *P. vivax* to infect *An. aquasalis* and *An. darlingi* from patients in early stages of treatments with different antimalarial drugs. This new knowledge, not yet known in the literature, will have important implications for the epidemiology of the disease and future studies indicating how transmission occurs in the field.

Viral metagenomic analysis of mosquitoes *Culex* (*Cux.*) *chidesteri* collected in the Sao Paulo Zoo Foundation

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Culicidae are the main vectors of arthropod-borne viruses (arboviruses) of medical importance and may be hosts of insect-specific viruses (ISVs) which do not replicate in vertebrates. Much evidence suggests that ISVs interact with arboviruses and may affect mosquito vector competence. Target-independent assay such as metagenomics based on the next-generation sequencing (NGS) have been used for detection and characterization of arboviruses and ISVs, besides new viruses. In this work, the viral metagenomics of *Culex* (*Cux.*) *chidesteri* collected at Sao Paulo Zoo Foundation was performed. In this zoo, there are environmental conditions that can lead to the emergence of arboviruses, such as the presence of reservoir animals, vector mosquitoes, Atlantic Forest, large flow of animals from other locations by migration or transfer between zoos, in addition to extensive human visitation in the fourth largest zoo in the world. Eight locations were selected for collection where CDC light traps were installed close to the ground and in the canopy. The collections were carried out for 12 hours between afternoon and morning twilight. Culicidae were morphologically identified and grouped into pools containing 20 to 50 specimens according to the species and conditions of collection. Samples were subjected to NGS and BlastX was used to link the generated contigs with sequences from the RefSeq virus protein database. A total of 1736 specimens of *Cx. chidesteri* were collected at all collection points in the zoo. For a preliminary analysis of viral metagenomics, 401 specimens were selected from six collection sites (BA, C61, LG70, RE, R38, R113). Amino acid sequences related to 10 different viral families were found where, among the families of known ISVs, sequences related to the families *Iflaviridae* (BA, LG70, RE, R38, R113), *Nodaviridae* (BA, C61, LG70, RE, R38, R113), *Peribunyaviridae* (C61, RE, R38, R113), *Totiviridae* (C61 close to the ground), *Mesoniviridae* (R38 close to the ground) and *Luteoviridae* (R38 and R113) were found. Sequences belonging to three families comprising both arbovirus and ISV were also found: *Flaviviridae*, *Reoviridae, Rhabdoviridae*. Sequences related to the *Flaviviridae* family were found in C61 and R113. *Rhabdoviridae* sequences were found only in specimens collected close to the ground in C61 and RE. Sequences related to the *Reoviridae* family were found only in samples collected in the canopy in the RE. This work identified viral families found in Cx (Cux.) chidesteri mosquitoes collected in different vegetation extracts at the Sao Paulo Zoo Foundation, providing information on the relationship between mosquitoes, arboviruses, ISVs and the environment. Additional studies such as the investigation of ISVs in males and larvae should be carried out in order to identify the maintenance of ISVs in the studied sites. Supported by FAPESP (17/50345-5 and 18/16232-1).

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A review on the mosquito vectors of Flavivirus in Argentina

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Infections caused by flaviviruses are among the most prevalent communicable diseases in the world, with mosquitoes being their main vector. There are about 3,500 mosquito species, of which only a small proportion transmit diseases. Knowledge of the vectors of these viruses is crucial for understanding the ecology of flaviviruses transmission cycles in the world and, particularly, in Argentina. The aim of this work is to describe the diversity of mosquito-borne flaviviruses, to detail the vector species involved (with evidence of infection in nature and competence assays with positive results) or potentially involved (with only evidence of infection in nature or positive competence assays) and to analyze their presence in Argentina and, in particular, in Buenos Aires Province. The ICTV Report on Virus Taxonomy was used to obtain information of known mosquito-borne flaviviruses and, for mosquito species, an extensive literature search was conducted using several academic search engines. 208 papers were consulted. There are 20 mosquito-borne flaviviruses and 30 considering subtypes. Of these 30 subtypes, 22 show evidence of human infection and 9 have evidence of circulation in Argentina (Bussuquara, Dengue 1/2/4, St. Louis Encephalitis, West Nile, Ilheus, Zika and Yellow Fever viruses). According to the available literature, 242 mosquito species were reported as vectors (45 confirmed, 197 probable) from the genera *Aedes* (82), *Aedeomyia* (2), *Anopheles* (33), *Armigeres* (1), *Coquillettidia* (10), *Culiseta* (8), *Culex* (69), *Deinocerites* (2), *Eretmapodites* (4), *Haemagogus* (5), *Mansonia* (9), *Mimomyia* (4), *Orthopodomyia* (1), *Psorophora* (7), *Sabethes* (4) and *Uranotaenia* (1). Of these, 36 species are present in Argentina, and 20 of them in Buenos Aires Province (4 *Aedes*, 2 *Anopheles*, 2 *Coquillettidia*, 6 *Culex*, 3 *Mansonia* and 3 *Psorophora*). In addition to the 9 flaviviruses reported in the country, others could be potentially introduced in the near future due to the presence of postulated mosquito vectors. Competence studies is highlighted as a vacancy area as the role that many of the species with evidence of infection could play in transmission cycles is unknown. It is worth mentioning that local information is scarce, and this is very important since most of the studies are from other continents, where the viruses and mosquitoes' strains are different from those of Argentina, so the results are not entirely extrapolable. This information is a valuable input to generate a potential risk map of these flaviviruses in Buenos Aires Province.

Susceptibility of *Aedes aegypti* mosquitoes to infection by different lineages of ZIKV

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Zika fever is a viral disease that has generated great concern for public health agencies in several countries, especially in tropical regions such as Brazil. It is a re-emerging disease, so it is enough that some situations are favorable for it to cause a new epidemic. The Zika virus (ZIKV), which causes Zika fever, is an arbovirus belonging to the Flaviviridae family, and is transmitted to humans by the bite of mosquitoes of the *Aedes* genus, such as *Aedes aegypti* and *Aedes albopictus*. It is divided into two main phylogenetic lineages, the African and the Asian. During the Zika outbreak in Brazil in 2015, the isolated strains were identified as of Asian origin. Nonetheless, in a study published in 2020, for the first time, African strains were detected in the South and Southeast regions of the country. One of them was isolated from the *Aedes albopictus* mosquito and the other from a species of monkey, the *Alouatta guariba*. The different locations suggest that the African lineage may have been circulating in Brazil for a significant period. It is already well described that mutations acquired during the evolution of this Flavivirus can alter its virulence. Studies using African and Asian strains confirmed the phenotypic differences between these different strains and demonstrated, in vitro, the greater virulence of the African strain. Studies were also carried out using populations of Aedes aegypti infected with ZIKV of African or Asian lineage and demonstrated greater pathogenicity and shorter incubation period of African lineage viruses, greater vector competence in Aedes aegypti and greater lethality in mice also infected with African lineage. As a way to assess the risks of virus transmission in Brazil, we propose a study on the rates of infection, disseminated infection and vector competence of *Ae. aegypti* in transmitting African and Asian ZIKV strains. The objective of the project is to investigate the differences in *Aedes aegypti* infections originating in the city of Belo Horizonte/MG, in Brazil, with the African and Asian Zika virus strains. For this, *Aedes aegypti* females are divided into two groups: one group submitted to infection with African ZIKV and the other submitted to infection with Asian ZIKV. At 7, 14 and 21 days after infection (dpi) the females are dissected, separating the salivary gland and carcass, and RNA and qPCR extractions are performed from each sample. Our preliminary results demonstrate that *Aedes aegypti* infected with Asian ZIKV have an infection rate equal to or greater than *Aedes aegypti* infected with the African lineage, since the spread rate is always higher in the group infected with Asian ZIKV. These results suggest that, among the strains used in this project, *Aedes aegypti*, from the city of Belo Horizonte, are more apt to be infected with the Asian lineage. This study will assist in the prevention and risk assessment of possible new ZIKV epidemics in Brazil and expanding knowledge about virus-vector interactions in different viral lineages.

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Evaluation of the influence of anti-*SARS-CoV*-2 antibodies on the vaccine response in *Aedes aegypti* infection with arbovirus (DENV, ZIKV, CHIKV)

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Covid-19, which started as a rapidly spreading, virtually unknown disease, has caused a global public health emergency for the first time this century. The knowledge about this disease has advanced. However, there are still many gaps and a great scarcity of works involving the vector-virus-host relationship. In addition to being non-existent, studies addressing the passage of anti-Covid vaccine antibodies to insects, specifically *Aedes aegypti*, could help in control strategies given their numerous implications. The project's objective will be to evaluate the influence of anti-SARS-CoV-2 antibodies on the vaccine response in the infection of *Aedes aegypti* with arbovirus (DENV, ZIKV, CHIKV). People over 18 years of age, men and non-pregnant women, will be invited to participate in the study, and then the informed consent will be presented. Participants will attend FMT-HVD for collection before the 1st dose and after taking the 1st and 2nd dose of the anti-Covid vaccine. To assess the immune response of the volunteers, IgG and IgM ELISA tests for DENV, ZIKV and CHIKV will be performed, using the sample before the 1st dose. ELISA tests are performed to quantify SARS-CoV-2 neutralizing antibodies, using the sample before the 1st dose and after the 1st and 2nd dose. For the transmissibility test, a population of *Ae. aegypti* bred in the laboratory. C6/36 cells grown in Leibowitz-15 medium will be used for cell culture. The titration of the virus in the infection will be performed by quantifying an aliquot of the cell culture by RT-qPCR. As much as the fight against the vector is carried out for years, epidemics persist. Therefore, the additional methods developed in to expand the fight against the disease and for that, it is necessary to understand the virus-vector interaction.

Seroprevalence of Dengue, Zika and Chikungunya virus infection in an endemic city in Brazil

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Arboviruses are a diverse group of vector-borne viruses, many of cause significant human morbidity and mortality. In recent years, arboviruses' emergence and/or resurgence has represented a considerable threat to global health. The continued geographic expansion of dengue viruses (DENV), along with explosive outbreaks of chikungunya virus (CHIKV) and, more recently, zika virus (ZIKV) have served as reminders that new epidemics can arise at any time from this diversity. The study aimed was to evaluate the seroprevalence of anti-DENV, anti-ZIKV and anti-CHIKV IgM and IgG antibodies in samples from healthy volunteers from the city of Manaus, Amazon. People over 18 years of age were invited to participate in the study, and then, the consent form was presented. Participants attended FMT-HVD for blood collection. Subsequently, the sample was centrifuged at 3.000 RPM for five minutes and the serum was stored in a freezer at -20 °C. To evaluate the immune response of the volunteers, enzyme immunosorbent assay tests (Kit Capture ELISA) were performed for IgG and IgM of dengue virus, chikungunya virus and zika virus using the serum sample. And the microplate reader was used to detect and interpret the results through the absorbance data. With 19 serum samples collected, 100% (19) tested negative for IgM of all arboviruses. Overall, DENV IgG positivity was 84% (16), 26% (5) for ZIKV, and 5% (1) for CHIKV. It was observed that 5% (1) had infection by the three arboviruses and 21% (4) by dengue and zika virus. A high proportion of study subjects have antibodies against DENV (84%), which makes them susceptible to new infections. These results reinforce the variation in the frequency and distribution of arboviruses during outbreaks, increasing the importance of differential diagnosis to identify agents that silently co-circulate with major health-problem arboviruses. Furthermore, the lack of preventive measures and vaccination against known and emerging mosquito-borne pathogens, and the occurrence of unexpected clinical complications, have an enormous social and economic impact on affected populations.

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Characterization and classification of autophagy-related genes in the *Anopheles aquasalis* genome

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Malaria is a parasitic disease transmitted by mosquitoes of the genus *Anopheles* sp., has a major impact on global public health, and threatens the economy of a third of the world's population. In Brazil, *Plasmodium vivax* is the main causative agent of malaria, and *Anopheles darlingi* is the primary transmission mosquito, followed by another important vector, *Anopheles aquasalis*. The parasite/vector interaction is understood in several molecular, biological, and immunological aspects; moreover, the widespread infection of the vector and its ability to transmit the disease to humans is dependent on the induction of genes related to processes such as autophagy, apoptosis, innate immune response, vitellogenesis, and digestion. This study's objective was to annotate genes associated with the cellular process of autophagy in *An. aquasalis*, comparing through phylogeny the predicted genes with other anophelines. The documentary study was carried out in the Bioinformatics laboratory of the Carlos Borborema Clinical Research Institute at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado. The genome data were obtained from the NCBI (National Center for Biotechnology Information) database (NJHH00000000.1), sequenced by Illumina HiSeq sequencing technology. The genes of interest were identified by homology using the annotated protein sequences of An. gambiae as a query. The other Diptera amino acid sequences were downloaded from the VectorBase databases, where proteins with identity above 35% were selected. Multiple alignments were performed, and the MEGA-X program generated the phylogenetic tree using the neighbor-joining test with 1000 bootstrap replicates..The results showed 16 autophagy proteins for *An. aquasalis* and a total of 62 sequences from the four-anopheline species. The results are still being analyzed, but it can be seen that the autophagy genes are a conserved group with a majority of clades with single-copy orthologs, where *An. aquasalis* had the same amount of genes compared to that identified in *An. gambiae*, except for the duplication of the protein related to the ATG12 gene.

Evaluation of the effectiveness of In2care traps for the monitoring and control of *Aedes aegypti* in Brazil

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The presence of *Ae. aegypti* associated with the circulation of Dengue, Chikungunya and Zika virus leaves thousands of people exposed to the risk of these diseases. Large-scale outbreaks occurring in several countries, including Brazil. Control strategies are mainly focused on reducing the population density of vector mosquitoes using as the main tools the elimination of breeding sites and the use of insecticides (chemical control). These strategies showed were not sufficient to contain the epidemics. The application of very potent larvicides, such as pyriproxyfen, by mosquitoes themselves using disseminator stations has been shown to be important for the control of immature phases of mosquitoes, reducing the population of *Aedes aegypti*. The In2Care trap attracts *Aedes* gravid females from and serves as an egg sink, preventing any emergence of adult mosquitoes. In addition, it is effective for the spread of pyriproxyfen and *Beauveria bassiana* spores, and can significantly reduce the survival of *the Aedes* exposed. The aim of this study is to evaluate the efficacy of In2care traps *to control Aedes aegypti* using ovitraps and BG GAT traps as well as to evaluate the operability and acceptability of the In2Care trap. The study was carried out in a neighbourhood of Pará de Minas, Minas Gerais, where 260 In2care were installed and distributed over 28 hectares. In2care were evaluated in terms of their attraction to gravid Aedes females and the larvicidal impact obtained within the trap and in the breeding sites of mosquitoes around. Entomological surveillance was performed at the intervention site and at a similar control site to measure the impact on Aedes populations. BG GAT traps were used for monitoring adult females of Aedes. Auto dissemination efficacy with more than 95% emergency inhibition of larvae exposed to water from ovitraps placed around the In2Care traps. A decrease in Ovitrap Index and in eggs density was noted during this study. The In2care proved to be a trap of easy operation and with excellent acceptability on the part of field agents and population. The results obtained demonstrated that In2Care trap as a promising tool for controlling *Aedes aegypti.* Large-scale studies should be implemented to assess the effectiveness of this tool.

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Determination of the diagnostic dose of SumiShield 50wg for Brazilian anophelines

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Malaria is still a serious public health problem in the Brazilian Amazon region. An important strategy to reduce malaria transmission is to combat the vector, through the use of chemical insecticides. Pyrethroid insecticides are the most used in vector control, however, resistance to these products has already been detected in anopheline populations in several countries. For this reason, new compounds with different modes of action are gradually being introduced into malaria vector control programs. Sumishield 50WG is an insecticide whose active ingredient is clothianidin, a neonicotinoid, having been included in the list of prequalified vector control products by the World Health Organization (WHO) in 2017 for use in indoor residual spraying. The WHO recommends actions to monitor insecticide resistance, and the determination of the diagnostic dose (DD) for new insecticides is essential to know the susceptibility of vector populations and detect any changes in the susceptibility status of these vectors after the implantation of chemical tools. Thus, the objective of this work was to find the SumiShield DD for Brazilian anopheline populations. To this end, dose-response bioassays were performed using the WHO tube methodology with impregnated papers, according to the WHO protocol (WHO, 2017). The doses tested ranged from 0.031% to 1%. In all bioassays, females of *Anopheles aquasalis* 3 to 5 days old were used, and they were obtained from a lineage established in the laboratory for over 25 years and used as a standard comparison strain. Females were exposed to insecticides for 1 hour and mortality readings were done after 1, 24, and 48h. Results were subjected to Probit analysis and LC_{50} and LC_{99} values were calculated. The LC_{50} and LC_{99} were 0.12% (CI=0.113–0.143) and 1.12% (CI=0.86–1.54), respectively. To obtain the DD, we doubled the CL_{99} value, resulting in a DD of 2.24%. The dose found was then tested on *Anopheles darlingi* females, the main vector of malaria in Brazil. The females were obtained from a laboratory lineage and resulted in 100% mortality. With the determination of this dose, we hope to have taken the first step towards monitoring the resistance of Brazilian anophelines to the new insecticides.

Risk of Yellow Fever Virus transmission in northern Argentina. Studies of vectors in sylvatic areas

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The Arbovirus represents an ecological based grouping on vector transmission by arthropods, between vertebrates (hosts) and hematophagous arthropods (vector) such as mosquitoes, simulids, sandflies and ticks. These viruses have great impact on public health, because causing sporadic and epidemic outbreaks that affect to human and animal populations. An important member is yellow fever virus (YFV), which is the prototype member of the genus *Flavivirus* (family *Flaviviridae*), that affects about 200,000 people each year causing 30,000 deaths in populations that are not vaccinated. During 2016- 2021 an outbreak of YFV affect Brasil, conferring an alert in the Northeast of Argentina. In this framework, we captured specimens in sylvatic areas form province of Misiones, Argentina. The captures were made during March and December 2019, in areas where there were populations of the black and golden howler monkey (*Alouatta caraya*). Mosquitoes were captured manually using entomologic nets at ground level. Insects collected in the field were placed in labeled vials, frozen in liquid nitrogen and shipped to the Instituto Nacional de Enfermedades Virales Humanas (INEVH-ANLIS) laboratory. At the laboratory, the specimens were sorted under a stereoscopic microscope on a chilled table by species, location and date of collection. Pools of mosquitoes were performed with the same species, same site and date of captured. Pools of mosquitoes were macerated in order to obtain a mosquito homogenate suspension and RNA extraction was performed with Trizol technique (TRI REAGENT TM), following by qRT-PCR for YFV and RT-Nested-PCR) for detect *Flavivirus* and *Alphavirus*. A total of 1084 specimens of mosquitoes (160 pools) were analyzed. Some of the captured species belonged to the genus: *Aedes*, *Haemagogus*, *Sabethes*, *Psorophora*, *Trichoprosopon, Wyeomyia,*The result of virological analysis was negative for YFV. However, some specific insect viruses were detected It is very important to continue with entomo-virological surveillance studies to detect circulation early of Arboviruses and to recognize new viral agents that could be circulating in the region.

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Sterile insect Technique (SIT) for *Ae. aegypti* control: The effect of Gamma radiation on the quality, sterility and mating competitiveness

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The *Ae. Aegypti* population and the vectorial transmission risk increase in Latin America each year, because of this, it is necessary to include in the pest management programs alternative tools for a vector control. SIT is one of the alternative strategies; this pest control technique was implemented in the past to control different insect species, with a good result. The aim of these trials was to evaluate the effect of gamma radiation on *Ae. Aegypti* males and females. Different doses were tested, 0 (Control),50,55,65,70,85and 110 Gy. During this trial the survival and the percentage of emergency(%E) were determined. These variables did not show significant difference between the control and the rest of the treatments. Doses of 50 and 70 Gy were chosen to determine the number of eggs laid, the hatch rate (HR) of these eggs for females mated with males from different treatments, the possibility of female remating after mate with an irradiated male and the sterility reversion of males. The HR showed significant differences between the eggs from no irradiated males (88.99±12.11%) and the eggs from irradiated males in dependent of the doses (50Gy: 0.93±1% and 70Gy: 0.79±1.55%). The female didn't remate after the first mate with the control and the irradiated male. The male didn't recover the fertility after the irradiation. The minimum dose to achieve 99% of sterility was 50 Gy. With this dose the female was irradiated. The %E and the number of eggs laid for the female irradiated and not irradiated were determined. The %E didn't show significant different between not irradiated females and irradiated. The females that were irradiated didn't lay any eggs, but the not irradiated laid 41 eggs per female. The competitiveness test index was 0.14 when 5 sterile male were released per each 1 fertile male (5:1) and this index increased to 0.54 when 10 sterile male were released per each fertile. The HR was 58.59% in the 5:1 cages and in the cages with 10:1 the proportion was 23.5%. These data are promising for the purpose of considering the incorporation of SIT in *Ae. Aegypti* as a tool for the integrated management of this vector.

Identification of metabolomic profile of mosquitos *Anopheles* diet by HPLC-MS technique - Lima Perú

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Malaria is an infectious disease transmitted by mosquito vectors of the family Culicidae of the genus *Anopheles*. In Peru, the distribution of cases is present in the tropical zones of the jungle and coast regions, the Centro Nacional de Epidemiología, Prevención y control de enfermedades del Ministerio de Salud to week nine reported 3,354 cases of *P. falciparum* and *P. vivax*, being Loreto, Junín, and Amazonas regions the more affected; these reports allow us to be attentive to possible outbreaks of Malaria in Lima region; since 5 cases of malaria have also been reported between 2018 and 2019 Malaria is a disease that ranges from mild to severe symptoms that can cause death, public health concern, economic and social problem, due to the proliferation of mosquitoes in areas with riparian basins. In Peru, the research about the nectarivores diet is limited and does not exist data about the metabolomic profile of the Anophelines species. The mosquito's crop is part of the digestive system, which plays an important role as a reserve of sugars from the various sources of floral nectars. Therefore, the metabolomic profile analysis of the mosquitos' crop is an important research factor; since the female and male mosquito seek different sources to feed such us carbohydrates among other nutrients to meet their physiological needs as part of their diet, these nutrients will be stored both in the crop and the midgut of the mosquitos. The objective of the project is to identify and create a database of the metabolomic profile of the diet feed of the Anophelines species in the riparian basins of the Lima region. The samples are the crops of the mosquitos An. *pseudopunctipennis* and *An*. *Albimanus* collected in the field, and the crops of the An. *Pseudopunctipennis* rearing in the insectary lab that has been feeding with five plants (plants (*Bidens alba, ladwigia octovalvis, ludwigia peploides, bougainvillea spectabilis and lonicera japónica*) with inflorescence collected in the field too; since methanolic extracts of the five plants and control quality internal standards; the metabolomic profile method is the qualitative instrumental analysis by the High Performance Liquid chromatography-mass spectrometry, the data analysis is performed by the software and libraries such us MZmine 2.51, Xcalibur 3.1, XCMS, Rstudio 4.2, and metaboanalyst 5.0. Two types of Anophelines were identified as *An. pseudopunctipennis* and *An*. *Albimanus*. The results obtained show that there is a relationship between the compound of plants and compounds found in the mosquito´s crop. Some compounds found are sugars (glucose, fructose, sucrose, and other sugars), amino acids, flavonoids, fungicides, pesticides, herbicides, and other natural compounds from the plants. The identification of the principal plants that have a major contribution to statistical analysis and their relationship with different compounds is essential for future research.

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Evaluation of the efficacy of oviposition traps with attractants in the population control of the *Aedes aegypti* mosquito

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The *Aedes aegypti* mosquito species is the main transmitter of the dengue virus in the region and is a vector of other viruses such as Zika, yellow fever and chikungunya. These diseases have increased their incidence over the last three decades and represent a growing health problem. There are several methods to control the vector's population level, the most widespread and considered the most effective is the elimination of potential oviposition sites. The use of ovitraps with the addition of compounds to lure gravid females to lay eggs is one of the alternatives currently under study. The objective of this work is to do a preliminary field evaluation of the efficacy of potential attractant solutions in ovitraps as instruments -complementary to eliminate oviposition sites-for the population control of the vector. For this purpose, two compounds reported in the literature as attractants were used: a mixture of yeast, sugar and water (0.5% m/v yeast and 28% m/v sugar) and a solution of *Paspalum dilatatatum* (0.4% m/v) in water, which were compared with a distilled water blank. The experiments were carried out in field at six sampling sites in the locality of Los Polvorines (Malvinas Argentinas, Buenos Aires). Ovitraps were placed at each site, three for each treatment and three for the distilled water blank. The traps were replaced every five days and the number of eggs oviposited per treatment per site was counted for a total of eight samplings. Preliminary results indicate that the distilled water control treatment was more effective in attracting mosquito oviposition than *Paspalum dilatatum*, while yeast with sugar and water solution did not show oviposition activity. In the case of treatment with *Paspalum dilatatatum*, a possibly explanation for the result could be that the pieces of the material prevent the reflection of light, which stimulates the females to locate the oviposition site. The carbon dioxide, on the other hand, released from the yeast and sugar solution is reported as a feeding attractant, which was tested as an oviposition attractant because of the specific attraction it produces in the gravid females and because the yeast would increase the chances of survival of the larvae. However, in the field it attracted other insects known to feed on mosquito eggs. Finally, it is important to continue with field studies of this type. Although the present study presents preliminary results, these trials sustained over time could demonstrate a promising option for the control of *Aedes aegypti* mosquitoes.

A field experiment to test the effect of containers brushing and scrubbing on *Aedes aegypti* eggs and non-target accompanying dipterans

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Aedes aegypti populations survive long periods of drought and winter temperatures in the egg stage, which remain attached to the walls of the containers until favourable conditions occur. Cleaning of water storage containers may not eliminate all immatures and cleaning techniques commonly used may lack the specificity needed to effectively remove *Ae. aegypti* eggs. More extended ovicidal procedures such as "the untadita" and its modifications requires concentrated chemicals (chlorine bleach and/or detergent), which not always are accepted or available in the entire community. In addition, there is a constant need to search for environmentally friendly control methods of *Ae. aegypti*, including the preservation of the accompanying fauna in the aquatic habitat. In this work, we compare the efficiency of two non-chemical methods, brushing and scrubbing, to remove *Ae. aegypti* eggs and the collateral effects on non-target dipterans that lay eggs on container walls. A field experiment was carried on during March 2022 on pre-existent and standardized flower vases (conic, plastic, black, 300cc) from Benavídez cemetery, Buenos Aires, Argentina. On three groups of 70 vases each, the following treatments were randomly applied: BR) brushing with toilet brush, SC) scrubbing with an all-purpose microfiber cloth and CT) control without intervention. In all the treatments, the water contained in the vase was previously emptied to eliminate pre-existing immatures and, after the application of the treatment, the vase was filled with tap water up to the top to promote the hatching of the remaining eggs. In two consecutive samplings carried out every 5 days, the content of each vase was checked to record and extract all *Ae. aegypti* immatures and non-target dipterans. The proportion of infested vases (vases with immatures/vases with water) was compared among treatments with Chi^2 and Tukey tests. The proportion of infested vases of *Ae. aegypti* was significantly lower in scrubbing than in brushing and control vases (SC: 3/126, BR: 16/129, CT: 26/128; $X^2_{(2)}$=19,99, p<0,0001; SC<CT, p<0,001; SC<BR, p<0,01). On the other hand, the proportion of infested vases with non-target Diptera was lower in both ovicidal treatments than in the control, both for the Psychodidae *Clogmia albipunctata* (SC: 0/126, BR: 0/129, CT: 5/128; $X^2_{(2)}$=7,07, p<0,029; SC<CT>BR, p<0,05) and the Ceratopogonidae *Dasyhelea necrophila* (SC: 0/126, BR: 3/129, CT: 15/128; $X^2_{(2)}$=21,92; p<0,0001; SC<CT, p<0,001; BR<CT, p<0,01). Scrubbing was the most efficient ovicidal method and reduced markedly the infestation levels of *Ae. aegypti* without the need of using chemical. Unfortunately, both ovicidal methods also had a strong impact on non-target insects with similar oviposition strategy than the dengue vector. In a wide-ranging control campaign there could be a drastic decrease in these non-target urban insects.

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An update on *Leptolegnia chapmanii* as control agent of vector mosquitos

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Leptolegnia chapmanii (Straminipila, Saprolegniales) is an oomycete known to be a specific pathogen of mosquitos (Culicidae) and blackflies (Simuliidae) larvae, being harmless to "non-target" aquatic organisms. Biology and ecology aspects of this microorganism are known, as well as the effect of abiotic variables, such as temperature, UV radiation and water quality, on its viability and efficacy as a control agent. The zoospores produced by *L. chapmanii* infect larvae through their cuticle or through digestive tract, when the larvae have ingested them. As an advantage, the handling of *L. chapmanii* under laboratory conditions is relatively simple, since it can be kept in nutrient culture medium. The presence of *L. chapmanii* has been reported in the United States, Argentina and Brazil, contemplating a possible cosmopolitan distribution. In Argentina it was found in the Province of Buenos Aires at the end of the 20th century, infecting larvae of the mosquito *Ochlerotatus albifasciatus*. Later, in 2015, it was reported infecting larvae of the yellow fever mosquito (*Aedes aegypti)* in the province of Misiones. More recently, in 2021, it was found in the National Natural Park "El Palmar", in Entre Ríos province, using floating traps with sentinel larvae. The taxonomic identification of this strain is corroborated by morphological characterization, molecular analysis, and pathogenic activity tests on *A. aegypti* larvae. This study contributes to expand the knowledge about the natural distribution of *L. chapmanii*. The isolation and identification of new strains of oomycetes would allow to expand the use of this microorganisms in microbial control strategies. Also, we are advancing in the development of a biological insecticide based on *L. chapmanii,* on a pilot and laboratory scale. In the biotechnological process, nutritional, economic and easily acquired substrates have been identified, viable for scaling up production. In cooperation with a private company, we are developing a massification, formulation, storage and application protocol. Furthermore, we are conducting efficacy studies under field conditions and safety studies in other "non-target" organisms. Surveys will be carried out soon in Misiones province (Argentina), and in Germany, in order to improve knowledge about the distribution of this entomopathogen, among other oomycetes of interest. We hope to expand the stock of biological material with potential for development, innovation and transfer processes to the public or private industry. The strains are deposited in the Mycological Collection at CEPAVE.

Development of a mosquito repellent formulation with an agroecological approach and socio-community impact

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The flight of mosquitoes constitutes a community concern, since they are a public health problem for transmitting viral diseases. The genera that abound in Argentina are *Aedes* and *Culex,* and the most common disease is Dengue. The management strategy involves interfering with the cycle of disease transmission by focusing on the vector insect, through preventive actions of environmental cleaning, chemical-rational control and repellents to avoid exposure and risk of bites. The proliferation of mosquito breeding grounds due to people's way of life increases their population density. Both insecticides and repellents available in the market are questioned for having adverse effects on health. The application of repellents prevents the possible transmission of diseases and allergic reactions, but it is not the main measure to take. From an agroecological perspective, it is proposed not only to carry out a participatory research methodology (PAR) in order to investigate the problem and the possible strategies to follow which involve the community as the main actor, but also to lower the incidence of dengue and to properly use the chemical products. The search for plant species offered by nature is also proposed. Its metabolites are not only effective, at a very low concentration, but are also biodegradable and low cost. Furthermore, they can be crafted to formulate an environmental flavoring or a repellent for daily use. The objective of this work was to design a comprehensive strategy to combat mosquitoes with an agroecological approach and a socio-community impact. Through surveys, the PAR methodology was used to relieve the knowledge that the community has about the behavior of mosquitoes, the form of transmission of dengue, the measures to prevent its proliferation and its control. A natural repellent mixture was formulated from *Cymbopogon citratus,* essential oil blends and mother tinctures of *Syzygium aromaticum* and *Lavandula burnatii*. Laboratory bioassays were carried out at three concentrations to evaluate the degree of repellency following the international protocol of the World Health Organization. The results of the surveys indicate that the community has a high degree of knowledge about the vector but a medium degree about the prevention of vector attacks, as well as a very good predisposition to replace the use of conventional repellents with others of natural origin. Greater knowledge of the mosquito from the community, effective intra-household measures and state management would help reduce the severity of the problem. For this effect, it is important to know about the life cycle of the mosquito, its behavior and the surfaces that act as breeding grounds, thus avoiding its proliferation. In order to promote change, it is also necessary to know the habits and customs of the people and the correct use of natural repellents. Educational actions and state intervention both in cities and in rural areas, teacher training at all levels are essential, as well, in order to raise awareness and achieve greater level of community engagement. Information is offered on trends in environmental management and the development of a natural product for immediate use necessary for health care.

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LEISHMANIASIS

Sandfly's (Diptera: Psychodidae: Phlebotominae: *Lutzomyia*) taxonomic diversity in a leishmaniasis endemic zone in northern Colombia

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The Montes de María region is one of the most important leishmaniasis macro hotspots in Colombia, with the municipality of El Carmen de Bolívar stands out for having high prevalence rates of visceral leishmaniasis. This municipality has fragments of tropical dry forest with abundant wildlife, incriminated as reservoirs of *Leishmania* and affected by fruit tree crops, providing optimal ecological conditions for the circulation of some phlebotomine sandflies species with epidemiological antecedents. Recently, 20 species of *Lutzomyia* were recorded for this municipality, which added to the presence of *Lu. evansi*, vector of *L. infantum chagasi*, etiologic agent of visceral leishmaniasis and *Lu. gomezi* and *Lu. panamensis*, vectors of *L. braziliensis* and *L. panamensis*, etiological agents of cutaneous leishmaniasis. As an aim, it was proposed to analyze the spatiotemporal variation of phlebotomine assemblage in El Bledo, El Carmen de Bolívar, a leishmaniasis endemic area in northern Colombia. Phlebotomine were collected during five samplings between January 2018 and April 2019, using CDC traps in the domestic, peridomestic and sylvatic ecotopes, during three consecutive nights/sampling, between 18:00 -06:00 h. *Lutzomyia* species were identified according to Young and Duncan (1994), taxonomic diversity and spatial variation was estimated, obtaining Hill numbers (richness q=0, typical species q=1 and abundant species q=2) in each ecotope with 95% confidence. A total of 8,784 individuals of the genus *Lutzomyia* distributed in eight species was collected, of which *Lu. evansi* (74.39%), *Lu. panamensis* (19.2%) and *Lu. gomezi* (6.31%) showed the highest abundances. According to the rarefaction/extrapolation curve based on sample size, the richness values for q=0 were not significant between peridomestic, domestic and sylvatic ecotopes, but if between domestic and sylvatic ecotopes, meaning that some species in peridomestic ecotope may have a wider range of distribution reflected in migrations between ecotopes. For orders q=1 and q=2, no significant differences were found, indicating that typical species were the most abundant in the ecotopes. Rarefaction/extrapolation curves revealed that in domestic and sylvatic ecotopes the greatest number of species was covered, while in peridomestic ecotope the number of possible species to be sampled was not reached, perhaps because some of them were cryptic. The accumulation curves per sampling revealed similarities in the diversities of the different ecotopes, in relation to the typical and dominant species present among them, with a notable decrease in richness during the first three samplings. The diversity orders in peridomestic ecotope remained constant in most of the samplings, varying only in the last sampling. In conclusion, the assemblages of the three ecotopes are similar (although the peridomestic ecotope has higher richness) because the typical and dominant species of the ecotopes are shared among them.

Life tables of *Lutzomyia longiflocosa* under semi-field conditions

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Phlebotomine sandflies are a group of insects of importance in public health, given their capacity to transmit pathogens, such as *Leishmania spp*. In Colombia, *Lutzomyia longiflocosa* is considered the most probable vector in cutaneous leishmaniasis outbreaks in the departments of Norte de Santander, Huila, and Tolima. However, several aspects of its biology and ecology are unknown, which directly affect it is role as a vector of the parasite. So it is necessary to know more about the characteristics inherent to its life cycle and life tables to make epidemic prediction models, and implement prevention of vector control measures against future outbreaks of the disease. In this study, life tables of *L. longiflocosa* were determined under semi-field conditions in Campoalegre, Huila, Colombia; the research was conducted using two cohorts of 100 individuals distributed in two time periods, in the drought period (semi-field 1) and rainy period (semi-field 2) between 2020 and 2021. Phlebotomine sandflies were collected using a Shannon trap between 18:00h-24:00h, then were transferred to a phlebotomine sandfly cage, and were fed with the help of an anesthetized hamster. The females were allowed to rest for several hours and the previously fed females were transported to plastic containers, next, they were organized in a Styrofoam cooler and it was placed in a work station at the study site. Survival, mortality, and number of individuals in each stage of development were recorded daily. Life tables were constructed with these data. The results for the cycle (semi-field 1) and cycle (semi-field 2) periods show high mortality in the egg stage with values of 35% and 23%, respectively. On the other hand, the cycle (semifield 2) showed higher mortality in the egg, larva 1, and larva 2 stages in contrast to the cycle (semifield 1) with values of 23%, 40%, and 26%, respectively; in addition, the instar with the lowest mortality was the pupa stage with values of 0.5% and 0.2% for each cycle, respectively. The larva stage 4 showed the longest duration with an average period time of 40 days and the adult stage showed the shortest duration with an average of 6 days. The average gonotrophic cycle was 9 and 11 days for each cycle respectively. Finally, the net reproductive rate (Ro) had values of 0.46 and 0.26 respectively for each cycle. This research represents an approach to the characteristics of the life cycle of *L. longiflocosa* in semi-field conditions, which will allow better understanding of the transmission dynamics of Leishmaniasis in endemic areas and will serve as a basis for further studies of eco-epidemiology.

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Genetic variability highlights the invasion route of the *Lutzomyia longipalpis*, the main vector responsible of Visceral Leishmaniasis vector in Uruguay

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In the Americas, the sandfly *Lutzomyia longipalpis* is the main vector of the parasitic protozoa *Leishmania infantum*, the etiological agent of visceral leishmaniasis (VL). The L*u. longipalpis* species complex is currently discontinuously distributed across the Neotropical region, from Mexico to the north of Argentina and Uruguay. During its continental spreading it must have adapted to several biomes and temperature amplitudes, when founder events should have contributed to the high genetic divergence and geographical structure currently observed reinforcing the speciation process. The first report of *Lu. longipalpis* in Uruguay was in 2010, calling the attention of Public Health authorities. Five years later the parasite *Le. infantum*, was associated with an outbreak of VL in canids. Hitherto seven human deaths by VL have been reported in Uruguay. They are presented here the first DNA sequences from the mitochondrial genes nad4 and cytb of *Lu. longipalpis* collected in Uruguay, and is evaluate its potential as molecular markers to investigate its genetic variability and population structure. We described four new nad4 haplotypes in a total of 98 (4/98) and one cytb in a total of 77 (1/77). As expected, we were able to establish that the *Lu. longipalpis* collected in two localities (i.e. Salto and Bella Unión) from the north of Uruguay are closely related to the populations from neighboring countries. We also propose that the possible route for the vector arrival to the region may have been through vegetation and forest corridors of the Uruguay River system, as well it may have benefited from landscape modifications generated by commercial forestation. The ecological-scale processes shaping *Lu. longipalpis* populations, the identification of genetically homogeneous groups and the gene flow among them, must be carefully investigated by using highly sensible molecular markers (i.e. genome wide SNPs) since it will help to the understanding of VL transmission and contribute to the planification of public policies on its control.

Diversity and geographic distribution of subfamily *Phlebotominae* in Uruguay

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Phlebotominae is a subfamily of insects distributed in the Americas, with more than 500 neotropical species. Some of them play a very important role as vectors in the transmission of different species of *Leishmania*, the causal agent of leishmaniasis. This work explores the diversity and geographic distribution of the different species of sandflies in Uruguay. The first studies on this subfamily date from the 1920s, the presence of two species of the genus *Lutzomyia* in our country is known, specimens of *L. gaminarai* having been found in the departments of Tacuarembó and Salto and, *Lutzomyia (Evandromyia) cortelezzi* in Montevideo. In 2010, the presence of specimens of *L. longipalpis* was reported for the first time in the north of the country, in the departments of Salto (city of Salto) and Artigas (city of Bella Unión). The geographical distribution of this vector has increased over the years from these first mentioned cities, always maintaining a spatial continuity. In 2018 the presence of *L. gaminarai* was reported in the department of Artigas and in 2019, the first specimens of *L. longipalpis* were reported in the city of Rivera. It is important to pay attention since this species has been implicated in the transmission of cutaneous leishmaniasis in neighboring countries (Brazil and Argentina).
Monitoring the distribution of sandfly species in different areas is important for the implementation of control programs aimed at reducing the risk of leishmaniasis infection.

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Genetic divergence in *Migonemyia migonei* (Diptera: Psychodidae) phlebotomine sandfly

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Migonemyia migonei is a zoophilic species with great adaptability to modified environments and also presents anthropophilic behavior. It has a wide geographical distribution from Colombia to Argentina, where it is the second species in abundance of records, covering 12 provinces. It is mainly associated with transmission cycles of cutaneous leishmaniasis, but it was also incriminated as a putative vector of *Leishmania infantum* (etiological agent of visceral leishmaniasis) in an enzootic cycle with accidental human transmission in Santiago del Estero, Argentina, in the absence of the primary vector *Lutzomyia longipalpis* complex. In Brazil, *Mg. migonei* is considered a permissive vector with demonstrated susceptibility to the development of different Leishmania species. Given its epidemiological importance, we analyzed the genetic divergence of the cytochrome C oxidase (COI) gene fragment from the sequences of 12 specimens collected in different localities in Argentina, and from 62 sequences available in Brazil, Colombia and Argentina. Genetic distances and variability were analyzed, a median-joining haplotype network constructed, and finally, a phylogenetic reconstruction was done according to Bayesian inference. The results suggest the existence of at least two monophyletic genetic lineages, one with the sequences of specimens from

Brazil, Colombia and Argentina, and the other with sequences only from Argentina. Interestingly, the sequences of specimens captured in sympatry in Misiones, Argentina, correspond to different lineages, which could indicate that this divergence is not due to geographic isolation. The results support the hypothesis of intraspecific genetic divergence, suggesting that *Mg. migonei* may be a species complex with at least two distinct lineages that could differ in traits of medical interest such as anthropophilia, adaptability, resistance to insecticides or vector competence.

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TICK-BORNE DISEASES (*Rickettsia*, *Ehrlichia*, *Borrelia*)

First report of *Haemaphysalis juxtakochi* ticks in marsh deer (*Blastocerus dichotomus*) from Argentina

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Vector-borne diseases represent 17% from whole infectious diseases and are responsible for more than 700.000 human deaths per year in the world. In this context, vector-borne disease drivers, including climate change, land use and land cover modifications, may contribute to higher incidence and wider geographic spread of vectors and vector-borne emerging pathogens. The recent emergence of several pathogens has highlighted the importance of wildlife disease surveillance for biodiversity conservation and global health. In the wetlands of northeastern Argentina, extraordinary floods have occurred frequently in recent years, including wildlife mortality events as for marsh deer (*Blastocerus dichotomus*). In this context, we have detected *Theileria cervi, Trypanosoma theileri, Trypanosoma evansi, Ehrlichia chaffeensis, Anaplasma platys, A. marginale, A. odocoilei* and Candidatus *Anaplasma boolense* in dead marsh deer. However, little is known about the wild transmission cycle of these pathogens in Argentina and the vectors involved have not been identified. High tick loads are frequent on marsh deer, and the species commonly found in individuals from argentine wetlands are *Amblyomma triste* and *Rhipicephalus microplus. Dermacentor nitens* and *A. cajennense* have been found feeding from marsh deer in the Brazilian Parana River region. As part of the wildlife health surveillance carried out since 2016, during 2019 we sampled nine marsh deer found dead in the Lower Delta of the Parana River. A fawn male of marsh deer with a high tick burden was reported. A total of 40 ticks were collected manually using acarological tweezers and stored in tubes containing 70º alcohol. The arthropods were taxonomically identified using a stereoscopic microscope (10X-40X, Nikon SMZ-2T) and taxonomic keys. All the 40 specimens corresponded to the species *Haemaphysalis juxtakochi* (18 adult males, 17 adult females and 5 nymphs). *Haemaphysalis juxtakochi* has been found parasitizing *Mazama americana, M. gouazoubira, M. nana, Ozotoceros bezoarticus* and *Axis axis* among other wild and domestic species in South America. In Argentina, the invasive species *A. axis* and cattle compete for territory with marsh deer, which means that these species could also be exposed to the same tick species. In this work we report the first finding of *H. juxtakochi* in marsh deer sampled in the Lower Delta of the Parana River.

Ehrlichiosis and anaplasmosis in dogs from Metropolitan Area of Buenos Aires. 2012-2021

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Tick-borne pathogens are widely distributed worldwide and are relevant in both human and animal health. In Argentina, two bacterial pathogens have been detected in dogs, both transmitted by the tick *Rhipicephalus sanguineus* sensu lato: *Ehrlichia canis*, agent of canine monocytic ehrlichiosis (CME), and *Anaplasma platys*, cause of canine infectious cyclic thrombocytopenia. The Metropolitan Area of Buenos Aires (MABA), comprising Buenos Aires City (BAC) and 40 municipalities of Greater Buenos Aires (GBA), has a large canine population and the presence of the vector. This work aimed to describe the molecular diagnosis of the genera *Ehrlichia* and *Anaplasma* (Anaplasmataceae family) in dogs with clinical signs from MABA. Samples from dogs with compatible signology were submitted to the Laboratorio de Vectores y Patógenos de Transmisión Vectorial of the Instituto de Zoonosis Luis Pasteur during the decade 2012-2021. DNA extraction was performed from blood with EDTA using two commercial kits. Multiplex PCR (polymerase chain reaction) was performed to amplify a 16S rRNA fragment from the Anaplasmataceae family, and a beta-actin gene fragment used as an endogenous control. *Anaplasma centrale* was used as positive control and nuclease-free water as negative. Analysis of the products was carried out in 1.5% agarose gels, stained with DNA intercalant and visualized in a UV light transilluminator. Absolute and relative frequencies of positive dogs by year and place of origin (GBA or BAC) were calculated. Between 2012 and 2021, 5,460 samples from MABA were studied (3,444 from BAC and 2016 from GBA). PCR results showed that 17.7% of the total samples were positive (12.2% for BAC and 27.1% for GBA). Positivity over the decade ranged from 7.7 to 23.9%. In 2017, the demand for diagnosis increased to 1,429 samples per year. Therefore, starting in 2018, requirements for sample acceptance were implemented, including compatible signology, thrombocytopenia and/or positive serology for *E. canis* and history of tick infestation. During 2020 and 2021, following the COVID-19 pandemic, there was a decrease in diagnostic demand. In this study, higher percentages of positivity were found for the GBA area compared to BAC, which would be related to a greater abundance of the vector due the presence of more favorable environments for its development. Clinical disease by *E. canis* in the studied area is widely confirmed; however, it is still necessary to optimize the diagnosis by combining a correct anamnesis, clinical examination, complementary laboratory and rapid serological techniques, and confirmatory techniques such as PCR.

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Distribution, transmission, and risk factors of infection with canine vector-borne pathogens in wild foxes and free-ranging dogs in Chile

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Canine vector-borne pathogens (CVBP) distribution tends to be circumscribed to certain areas with climatic features where their vectors are able to persist. In this aspect, Chile possesses a variety of bioclimates that provides an ideal scenario to study the distribution of vector-borne pathogens, considering that ticks of the *Rhipicephalus sanguineus* species group are widely distributed in the country. Despite this, information about presence, distribution, and impact of CVBP in domestic and wild canines in Chile is scarce. In this study, blood and serum samples of 981 dogs, 155 Andean foxes (*Lycalopex culpaeus*), and 90 grey foxes (*L. griseus*) were obtained from six different bioclimatic regions throughout Chile. Molecular tools and serological tests were used to detect presence or exposure of CVBP. Ticks retrieved were identified using taxonomic keys and molecular tools, finally, risk factors of infection were inferred. The occurrence *Anaplasma platys, Mycoplasma haemocanis,* and *Candidatus Mycoplasma haematoparvum* were confirmed in the three canid species and in all six bioclimatic regions with presence of *R. sanguineus. Ehrlichia canis* DNA was not detected in any sample. *Hepatozoon felis, H. americanum,* and *H. canis* were confirmed for the first time in foxes of Chile. *Babesia vogeli* DNA and antibodies were found in dogs, while in foxes only antibodies against this agent were detected. No filariids other than *Acanthocheilonema reconditum* were confirmed in our samples. Our molecular results support the hypothesis that interspecific transmission is taking place, and that free-ranging dogs in Chile could be favoring the maintenance of some CVBP in areas suitable for their tick vector. Due to the comprehensive nature of this work, in which the most relevant CVBP in canid species of Chile were studied across a latitudinal gradient of 3000 km, this project contributes to fill the knowledge gap in the epidemiology of vector-borne pathogens shared between domestic and wild animals, having implications in the transmission ways of the studied agents.

Potential urban transmission focus of Rickettsiosis in Merida, Yucatan, Mexico

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Yucatan has had a continuous report of cases of rickettsioses, records of infected synanthropic, domestic animals and ectoparasites in rural and urban areas. The present study aimed to describe how the occurrence of infected mammals and their ectoparasites interact in human urban households and which characteristics are associated with the presence of *Rickettsia* infected animals. Synanthropic mammals were captured in selected households, and we took a whole-blood sample from each animal. For the dogs, we obtained the owner's authorization and collected 1-5 ml of whole-blood. All animals were physically inspected for direct ectoparasite collection. Molecular and serological analysis were performed by PCR and IFA. We applied a questionnaire to an adult householder for variables that describe the conditions and characteristics of the property. A total of 99 mammals were sampled, 29 opossums, 13 house mice, 7 black rats and 50 dogs. Infection occurrence in opossums was 8.3% of Spotted Fever Group (SFG), 50% for Typhus Group (TG), and 4.2% undetermined. House mice 46.2% for SFG and 30.8% were undetermined. Black rats 28.6% of SFG and 57.1% undetermined. Finally, dogs were 19.1% of SFG, 57.4% to TG, and 23.4% undetermined. A total of 424 ectoparasites were collected from the mammals. In opossums occurred the ticks *Ambyomma spp., Ornithodoros (Alecterobius)* nr. *talaje,* and the flea *Ctenocephalides felis*. In dogs we found the previous ones and *Rhipicephalus sanguineus* s. l. No ectoparasites were collected from rodents. The occurrence of infected animals was associated primarily with the material of the backyard floor, the type of sanitary system, the presence of garbage, stored firewood, PET containers, and construction supplies. A generalized linear model showed that the household with backyard with a dirt floor or other non-concrete material has more chances of harboring infected animals. In contrast, When the house has a sanitary system or a latrine outside the house, these chances decrease significantly. The results of this study show the ecological interactions that occur around the urban transmission of rickettsiosis in Yucatan. The evidence of pathogenic *Rickettsia* in vectors and host animals in the vicinity of households, shows the need for studies that specifically address the dynamics of longitudinal and spatial transmission between populations of these species and its relationship with the occurrence of people with rickettsiosis. Factors like the sanitary system show a clear relationship with the presence of synanthropic and domestic animals infected by *Rickettsia*. These findings suggest the presence of a potential transmission focus of Rickettsiosis in Merida and reveals the necessity to generate knowledge on the epidemiology of Rickettsiosis in Yucatan.

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First molecular report of *Candidatus Rickettsia andeanae* in wild and domestic host from Paraguay

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Rickettsiae are obligate intracellular Gram-negative bacteria that cause febrile zoonotic diseases with high lethality in cases of late treatment. They possess a global distribution and are transmitted by ticks, fleas, lice and mites, with ticks being the most important in veterinary medicine and public health. Within the Rickettsia species, *R. rickettsi* is considered the most pathogenic and has been reported in several South American countries. In Paraguay there are no reports of *Rickettsia spp* species circulating in wild or domestic hosts or their possible vectors. For this reason, the present study aimed to molecularly detect the presence of *Rickettsia spp* in wild and domestic animals and their ectoparasites (ticks) collected in the Reserva de Biosfera del Bosque Mbaracayú (RBBM), Canindeyú Department, Paraguay. 175 DNA samples were analyzed belonging to *Canis familiaris*, *Dasypus novemcinctus* and ticks collected from the host. The samples were collected in 2017 in the RBBM. The genetic material was extracted using a commercial kit following the manufacturer's instructions. The presence of *Rickettsia spp* DNA was detected by real-time and conventional PCR using the specific primers gltA and OmpA. The resulting products were sequenced and edited with BioEdit software, afterwards a similarity search was performed using the GenBank database to determine the species. Of the 175 samples analyzed, 110 were from whole blood and 65 from ticks. The blood samples were 89 from *Canis familiaris* (31 females and 57 males) and 21 from *D. novemcinctus*. Regarding the ectoparasites, 24 were from dogs (8 nymphs and 16 adults of *Rhipicephalus sanguineus*) and 41 ticks were collected from *D. novemcinctus* (2 nymphs of *Amblyomma coelebs*, 18 nymphs and 21 adults of *Amblyomma sculptum*). The presence of *Rickettsia spp* was detected in 15 (8.6%) of the analyzed samples. Being 4 (2.3%) for *Canis familiaris*, 5 (2.9%) for *D.novemcinctus* and 6 (3.4%) in ticks. It was obtained, in all the analyzed sequences, a similarity percentage equivalent to 99% identity with the sequences of *Candidatus Rickettsia andeanae* deposited in the GenBank. The results indicate a possible silent circulation of *Rickettsia spp* in the wild and in areas adjacent to the Mbaracayú-Paraguay reserve. The epidemiological scenario is aggravated by the presence of dogs that would act as reservoirs and that in many cases enter the reserve exposing the surrounding community and wildlife. The molecular detection of the bacterium *Candidatus Rickettsia andeanae* in domestic and wild hosts is reported for the first time for Paraguay. Although the pathogenicity of this species is still unknown, research in the country should be increased to elucidate the epidemiological behavior of the *Rickettsia* pathogen-vector-host relationship in Paraguay and its possible implications for public health.

Ticks in periurban areas from the municipalities of La Costa and General Lavalle. A preliminary study.

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In Argentina, spotted fever rickettsiosis caused by *Rickettsia parkeri* is a zoonotic disease with tick *Amblyomma triste* as its principal vector. The presence of this tick species restricts to areas of La Plata hydrographic basin in the province of Buenos Aires. The adult stages of *A. triste* can parasitize dogs and cats. The aim of this study is to detect the presence of *A. triste* in dogs and humans from municipalities of General Lavalle and La Costa (Buenos Aires). The study period was between September and December 2021, the period of the main activity for adults of *A. triste*. A total of two dog´s shelters in peri-urban areas were surveyed: one private (General Lavalle, municipality of General Lavalle) and another public (Mar del Tuyú, municipality of La Costa). In adittion, a citizen science scheme was proposed inviting the veterinarian to report tick samples obtained in their clinical practices and also the community was persuaded to collect ticks found on dogs and humans. The collected specimens were sent to the Laboratorio de Vectores y Patógenos de Transmisión Vectorial of Instituto de Zoonosis Luis Pasteur for taxonomic identification. Forty-nine dogs were sampled, 21 from shelters (15 from General Lavalle and 6 from La Costa); and 28 referred by private veterinarians and the community (8 from General Lavalle, and 20 from La Costa). In addition, specimens were collected from 4 humans from Las Toninas and Mar del Tuyú (municipality of La Costa). The presence of *A. triste* was confirmed in 20.4% of the sampled dogs, while *Rhipicephalus sanguineus* sensu lato was detected in the remaining 79.6% of the cases (14.3% and 32.6% for Gral. Lavalle; and 6.1% and 46.9% for La Costa, respectively). In total, 213 adult specimens were collected from dogs, 24 were identified as *A. triste* and 189 as *R. sanguineus* s.l. (188 adults and 1 nymphs). The ticks collected on the four humans were identified as *A. triste* in 3 cases (12 adults) and *R. sanguineus* s.l. in the remaining case (one adult specimen). This preliminary study has documented the presence of *A. triste* associated with dogs in peri-urban environments of the municipalities of La Costa and General Lavalle, as well as the occurrence of parasitism in humans. It is worth to note the importance of collaborative work with the community and private professionals when surveying areas not yet studied.

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Fleas in wild mammals: first records of *Ctenocephalides felis-Rickettsia felis* complex in Patagonia

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Adult fleas (Insecta: Siphonaptera) are blood-sucking parasites of birds and mammals. Also they are known as vectors of several pathogens of important diseases in animals and humans. The aim of this study was to evaluate the diversity of fleas and the presence of *Rickettsia* spp. in wild native mammals to the extreme northeast of Río Negro province, Argentine Patagonia. Between 2020 and 2021 fleas were collected from 12 hosts of four orders and five species. Order Didelphimorphia: three white-eared opossum (*Didelphis albiventris*), order Cingulata: two big hairly armadillo (*Chaetophractus villosus*), order Carnivora: three south american grey fox (*Lycalopex gymnocercus*) and one geoffroy's cat (*Leopardus geoffroyi*) and order Rodentia: three plains vizcacha (*Lagostomus maximus*). The animals were found lifeless due to being run over. They were immediately placed in a white polyethylene bag to facilitate the collection of fleas. A total of 154 fleas were recovered that were preserved in 96% alcohol. Total genomic DNA was extracted from 26 fleas collected in the 12 infested mammals using the Chelex resin protocol. The presence of *Rickettsia* was screened using conventional PCR for three gene fragments, *gltA* (610 bp), *ompB* (650 bp) and *ompA* (580 bp). The following associations (host-flea) were identified: *L. maximus - C. felis*, *L. geoffroyi- C. felis*, *L. geoffroyi- Pulex irritans*, *L. gymnocercus- P. irritans*, *L. maximus- P. irritans*, *L. maximus- Hectopsylla* sp., *C. villosus-Phthiropsylla agenoris*. Also, a Rickettsial bacterium was detected in a single flea sample, *C. felis,* collected from a female synanthropic white-eared opossum. The nBLAST analysis of the three gene fragments in the positive sample showed identity 100% with *R. felis.* This study contributes to knowledge of the biodiversity from Patagonia and provides novel information about flea-pathogen associations in wildlife and represents the first records of *C. felis- R. felis* complex in Patagonia. Also, there is the first record of *L. geoffroyi* as host of *C. felis* and *P. irritans*. *R. felis* (causative agent of flea-borne spotted fever) is one of the emerging human rickettsiosis agents worldwide. This result suggests the need to replicate and increase this type of studies in the wild fauna of Patagonia in order to determine the possible presence of an epidemiological network implicated in the circulation, transmission, and the maintain of *R. felis* in wild animals and their fleas.

Survey of ixodid ticks, vectors of *Rickettsia parkeri*, Buenos Aires province, Argentina

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In America, rickettsiae have aroused greater interest in public health. Categorized as emerging zoonotic diseases called "Spotted Fever" (FM), this group of diseases are transmitted to humans essentially by hematophagous arthropods such as Ixodidae ticks. This problem exists in Argentina, where the main vector of the *Rickettsia parkeri* bacterium (within FM), in areas of the Plata Hydrographic Basin, is the hard tick of the *Amblyomma triste* species. The aim of this study is to survey the species of ticks, mainly of the genus *Amblyomma spp*., both in urban (UA) and natural (NA) areas, in municipalities that are part of the Plata River basin and its sub-basins, in order to make new contributions to its epidemiology and surveillance, during the period from September 2021 to March 2022. A descriptive, quantitative, observational and cross-sectional study was carried out to survey the different species of ticks in NA and UA, in the Plata River Hydrographic Basin. The collection of specimens in NA was carried out using the flag method, while in UA they were obtained from dogs and cats that were taken to the corresponding Zoonosis Centers or to Veterinary Clinics. A total number of 3524 specimens were obtained: 1844 belonged ticks of the genus *Amblyomma spp.* (90.58% adults, 0.22% nymphs, both *A. triste*; 9.20% larvae, in which only the genus could be identified); and 1680 to *Rhipicephalus sanguineus* only in UA (in canines and/or felines). *A. triste* was the only species found in NA, but it was also detected in 2.27% of the ticks found in UA, in the municipalities of San Nicolás, La Plata, General Lavalle and La Costa, attached to domestic animals or people, mainly related to transition strips with NA. No specimens of *A. tigrinum* (another *Rickettsia parkeri* vector) were recorded. Based on these results, we can conclude that within Buenos Aires province, the population with a high risk of contracting FM is the one found in areas where the vector, *A. triste*, is present. NA are the ones with the highest zoonotic risk, with beingpark rangers or researchers being the most exposed social group, but also the different anthropic activities, bringing man closer to these zoonoses. Given the emerging nature of tick-borne infections, it is necessary to continue with ecoepidemiological studies of FM, design new strategies for active surveillance and aware health teams about rickettsioses with a multidisciplinary focus, understanding health as "One".

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VIRAL HAEMORRHAGIC FEVERS (HANTAVIRUS)

Identification of possible reservoirs of *Mammarenavirus* in the Cerrado biome of Minas Gerais, Brazil

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Members of the family Arenaviridae, genus *Mammarenavirus* are associated with hemorrhagic fevers and neurological impairments in humans and have rodents of different species as their main natural reservoirs. So far, eight species of mammarenaviruses have been described in Brazil in ten different species of wild rodents, except for Sabiá virus (SABV), the etiological agent of Brazilian hemorrhagic fever (BHF), whose natural reservoir is still unknown. In December 2019, BHF and which re-emerged in the State of São Paulo, reinforcing the need for investigation to detect possible reservoirs of SABV and other mammarenaviruses in the country, especially in the Cerrado (savanna-like) biome. This project aims to evaluate mammarenaviruses' seroprevalence in rodents collected in the Cerrado area of Minas Gerais - Brazil. Therefore, 260 samples of wild rodents were selected for serological analysis, collected between December 2011 and November 2012, in a longitudinal study carried out in the state of Minas Gerais, deposited at the Laboratory of Hantaviruses and Rickettsiosis, IOC/FIOCRUZ, Rio de Janeiro, Brazil. Rodents were captured using Sherman live-capture traps set in rural areas. Serological analysis were performed in serum and blood samples using a pre-established protocol, through an IgG enzyme immunoassay (ELISA) based on Junin Mammarenavirus specific antigen. So far, we have analyzed 80 samples of wild rodents, belonging to the following species: *Necromys lasiurus* (63), *Calomys tener* (5), *Calomys expulsus* (5), *Cerradomys subflavus* (3), *Cerradomus marinhus* (1), *Pseudoryzomys simplex* (1), *Oxymycterus delator* (1), *Oecomys sp*. (1). Mammarenaviruses antibodies were detected in one *N. lasiurus* (1.25%), the most prevalent wild rodent species in the Cerrado biome. The high abundance of these rodent species is favored by interspecific habitat competition in anthropogenic environments, due to changes that have occurred in this biome in recent decades. Recently, *N. lasiurus* specimens have been described as a reservoir of Oliveros (Arenaviridae: Mammarenavirus) in the state of Mato Grosso do Sul, approximately 825 km from our study site. Our data indicates that *N. lasiurus* can act as mammarenavirus reservoir in different areas of Brazilian Cerrado. This is the first report of mammarenavirus circulation in Minas Gerais State. The findings of this study highlight the need for more surveys on arenavirus in small rodents from different regions of Brazil.

Wenzhou (Arenaviridae: Mammarenavirus) Detection in Synanthropic Rodents in the State of Bahia, Brazil

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In recent decades studies of zoonotic diseases have focused on those transmitted to humans by wild fauna. Few studies have drawn attention to animals adapted to the urban areas, such as some synanthropic rodent species capable of adapting to anthropized environments. As an example, we can mention the species *Rattus rattus, Rattus norvegicus* and *Mus musculus*, which have a heterogeneous and cosmopolitan distribution, expanding their range as their interaction with humans increases. These animals are natural reservoirs of several pathogens and are involved in the emergence and spread of different viruses, bacteria and protozoa throughout the world. Recently, a new mammarenavirus, Wenzhou virus (WENV), was detected in samples of *R. norvegicus, M. musculus, Rattus flavipectus, Niviventer niviventer* and *Suncus murinus* from China, but its distribution outside the Asian continent remains unknown. WENV genome is composed of two segments of negative single-stranded RNA and is phylogenetically associated with viruses identified in Asia, Europe and Africa, including Lymphocytic Choriomeningitis virus (LCMV). Initial studies demonstrate evidence of human WENV infection in areas where the virus has been detected in their animal hosts. The aim of this work was to investigate the circulation of WENV in samples of *R. novergicus* rodents captured in Brazil. This study included samples from 51 rodents captured in four locations in the city of Salvador between 2010 and 2019. Total RNA was extracted from lung fragment samples and then submitted to RT-PCR followed by semi-nested for the amplification of partial fragment (302bp) of the WENV polymerase gene. One sample (1.96% - 1/51) showed amplification in the expected size and was submitted to sequencing for further confirmation. In Brazil, surveys carried out in regions of socioeconomic vulnerability in the cities of São Paulo and Salvador showed high rates of rodent infestation. Even so, there are relatively few studies that address the monitoring of viruses in these potential hosts in the country. Our study is the first evidence of WENV circulation outside Asia, demonstrating that, like its host, the virus probably has a cosmopolitan distribution. This data reinforces the need of more eco-epidemiological studies focused on synanthropic rodents, to support the implementation of surveillance actions and control and prevention activities of WENV.

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Hantavirus in rodents of Buenos Aires Province: are seroprevalence and abundance related?

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Oligoryzomys flavescens and *Akodon azarae* are two rodent species living in agroecosystems of the Pampean region. *O. flavescens* is a reservoir of the Lechiguanas genotype, associated with Hantavirus Pulmonary Syndrome, whereas *Akodon azarae* is a reservoir of the Pergamino genotype, which has not been associated with human cases. Our objective was to evaluate whether there is a relationship between abundance and seroprevalence in both rodent species, as this may help to identify situations of high risk of exposure to hantavirus for humans. Eleven longitudinal rodent capture-mark-recapture surveys were conducted in three railway embankments in agricultural landscapes (Exaltación de la Cruz Departament, Buenos Aires province, Argentina), from 2014 through 2016. The trapping effort was 1800 trap-nights per survey. During these surveys, demographic data and blood samples were collected. Blood samples were analyzed by means of ELISAs to determine the presence of hantavirus-specific antibodies. For each rodent species, the relationship between seroprevalence and its abundance was assessed through logit-linked binomial generalized linear models using the number of infected individuals by sampling session as the response variable (i.e., successees, with the corresponding number of tested blood samples per group as trials). Models containing the species' MNA as a predictor and the null models were evaluated. Using a multi-model approach, averaged parameters and their relative importance were calculated using Akaike weights (AIC). The main finding in this work was that both *A. azarae* and *O. flavescens* exhibit a negative relationship between prevalence and abundance. A possible explanation for this result is that populations reach their smaller numbers when these consist mainly of overwintering adults, which had longer exposures with higher chances of becoming infected, whereas larger populations are observed soon after the reproductive season, when new recruits are unlikely to be infected yet. Thus, the effect of prevalence and abundance on the risk of human exposure could be compensatory. This suggests that there would be no particular season of increased risk; prevention and surveillance should be permanent.

First evidence of circulation of the Alto Paraguay orthohantavirus genotype in Argentina: human case and reservoir rodent

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Hantavirus pulmonary syndrome (HPS) is an acute viral illness caused by members of the genus *Orthohantavirus*, family Hantaviridae. In Argentina, there is evidence of the circulation of several pathogenic viral genotypes and their respective reservoir rodents (Bermejo - *Oligoryzomys occidentalis*, Oran – *O. chacoensis*, Laguna Negra - *Calomys fecundus*, Juquitiba – *O. nigripes*, Lechiguanas – *O. flavescens* and *O. nigripes*, HU39694 – *O. flavescens,* Andes – *O. longicaudatus*) and two non-pathogenic genotypes for humans (Pergamino - *Akodon azarae* and Maciel - *Necromys benefactus*). In 2020, the diagnosis of a patient with HPS was carried out from Colonia Montefiore (Santa Fe province), without a travel history. A serum sample of the patient was analyzed by IgM and IgG ELISA for antibody detection. The viral genome was detected by RNA extraction from the serum sample followed by nested RT-PCR using generic primers that amplified fragments from the S and M genome segments. The orthohantavirus genotype present in the sample was Alto Paraguay. This genotype had previously been only detected in Paraguay in rodents identified as *Holochilus chacarius*, but not in patients with HPS. Since the circulation of this genotype was unknown in Argentina, with the main objective of identify the rodent reservoir of Alto Paraguay in our country, we developed a small and focused ecological sampling near dwellings in Colonia Montefiore. We captured 59 rodents with a sampling effort of 420 traps–nights, during three nights. An individual of *H. chacarius* infected with the Alto Paraguay genotype was detected. The viral sequence of the rodent was compared with that of the patient, finding 100% nucleotide identity for the S and M genomic segments. This result strongly suggest that *H. chacarius* would be the reservoir of Alto Paraguay in Argentina. Future studies should be conducted to determine whether the circulation of this viral genotype covers other areas of Argentina.

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LEPTOSPIROSIS

Detection of *Leptospira* excretion in Galapagos sea lions (*Zalophus wollebaeki*) using real-time PCR with TaqMan probe

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The Galapagos sea lion (*Zalophus wollebaeki*) is a species categorized as endangered by the IUCN, due to the drastic decline that has suffered its population in recent decades. Since *Z. wollebaeki* inhabits a hotspot of marine productivity amid a tropical environment, this species is exposed to frequent periods of low productivity (e.g. El Niño–Southern Oscillation ENSO) that generate food stress and increases its mortality rate. Another possible cause of mortality are infectious diseases; however, little is known about the pathogens that affect the health and population dynamics of this species. In *Zalophus californianus*, the closest relative of *Z. wollebaeki*, *Leptospira interrogans* serovar Pomona has been identified as the infectious agent responsible for high morbidity and mortality. In this species, outbreaks of leptospirosis have been observed every 3-5 years evidenced as asymptomatic infections and abortions. Recent studies detected *Leptospira* in samples of Galapagos sea lions; however, its epidemiology, prevalence, and circulating species are unknown. The aim of this study is to evaluate the excretion of *Leptospira* in the urine of sea lions in the El Malecón rookery, which is the largest *Z. wollebaeki* population in the archipelago. We were able to take samples in 8 of the beaches where this rookery rest. Due to the difficulties of taking a urine sample directly from these animals, sand samples with fresh urine were collected right after the animals urinated. As this was an exploratory study and to maximize the possibility of finding positive samples, sand pools were made with urine from 6 to 8 individuals. A total of 24 pools were collected, of which 10 were analyzed for preliminary results that are presented in this work. After extracting the DNA from each sample, a PCR was performed with TaqMan probes that detect a fragment of the *lipl32* gene present only in pathogenic *Leptospira* species. We found *Leptospira* DNA in 90% of pools analyzed, suggesting the excretion of bacteria in the urine of sea lions from the El Malecón. These results provide the first step towards understanding the ecoepidemiology of leptospirosis in the Galapagos sea lions. Future studies will be designated to understand the leptospira species that circulate among these animals, and the impact of infection on sea lion colonies in the islands. In addition, it is important to continue this research and understand if this pathogen circulates in other endemic species of the island or in domestic animals.

A look at leptospirosis in a rural area of Ecuador

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Leptospirosis is a disease endemic to countries with subtropical and tropical climates. It affects people working with animals or outdoors in places with large accumulations of water, such as rice fields or after extreme weather events. Leptospirosis is most common in rural areas of Ecuador, but limited information is known about the epidemiology of the disease in these areas. Our study was conducted in a rural community located in the province of Manabí, on the coast of Ecuador. The main aim of the study was to determine the exposure to leptospires and the excretion of the pathogen by humans and animals. For this purpose, eight houses where animals are reared and used mainly for family consumption, were selected in the community. Serum samples from animals and humans were collected and tested by microagglutination test (MAT) with 23 serovars of pathogenic *Leptospira*. In addition, urine samples were collected and analyzed by two Taqman PCR assays that detect the Lipl32 and 16S rRNA genes of pathogenic *Leptospira* species. The results of these assays showed that 100% of the cows, pigs and dogs had MAT titers greater than 1:100, indicating that all had been exposed to the pathogen. *Leptospira* DNA was detected in urine of a high percentage of these animals (7/11 dogs, 3/10 cows, 6/15 pigs), suggesting that these animals are excreting the bacteria in their urine. On the other hand, MAT results from human samples show that 47.82% (n = 23) of people have been exposed to the pathogen (titers between 1:100 and 1:200). *Leptospira* DNA was detected on 69.56% (n =16/23) urine samples of these individuals. Serological and molecular findings show that inhabitants of this community are highly exposed to leptospirosis and that pathogenic Leptospira DNA excretion is associated with male gender (Fisher exact test, p= 0.0251*): 12 male and 4 female positive samples. Moreover, high positivity found in animals suggests that exposure to pathogenic Leptospira is mainly due to the proximity of people to domestic animals and probably to animal husbandry practices. Further studies will allow us to learn about behaviors and practices that could be modified to prevent infection of people living in this community.

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Leptospirosis in Aguará Guazú (*Chysocyonbrachyurus*), an epidemiological challenge

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The leptospirosis is the most widespread zoonosis in the world. Knowledge about the prevalence of *Leptospira* serotypes in wild animals such as Aguará Guazú *(Chysocyonbrachyurus)* is a challenge to understand the epidemiological cycle of the disease, both in its natural environment or in captive conditions. In our country, few studies have been carried out on these animals. With the objective of knowing the seropositivity rate of leptospirosis, detecting the presence of leptospira DNA in serum samples from Aguara Guazú and to increase the epidemiological knowledge of this bacterium with its involvement in wildlife. Twenty-five serum samples (n=25) of clinically healthy Aguará Guazú housed in a Wildlife Rescue, Rehabilitation and Relocation Centre "La Esmeralda" (Santa Fe, Argentina) were studied. The Microscopic Agglutination Test (MAT), the international reference serological technique, was performed in sera to evaluate the seropositivity rate. The following serogroups/serovars were used: Ballum/ Castellonis, Canicola/ Canicola, Icterohaemorrhagiae/ Copenhageni, Pomona/ Pomona, Pyrogenes/ Pyrogenes, Tarassovi/ Tarassovi, Sejroe/ Wolffii, Sejroe/ Hardjo, Sejroe/ Sejroe, Bataviae/ Bataviae, Australis/ Australis, Autumnalis/ Autumnalis, Cynopteri/ Cynopteri, Hebdomadis/ Hebdomadis, Javanica/ Javanica and Panama/ Panama. Cut-off titre of 1:100 was considered, following the Argentinean Association of Veterinary Diagnostic Laboratories (AAVLD) recommendations. DNA extraction and partial amplification of *rrs* (16S rRNA) gene by TaqMan real-time PCR (Smythe 2002) was also performed in serum samples. A seropositivity rate of 72% (n=18) was evidenced in the samples studied. Titers against more than one serogroup were obtained in 17 samples. The most frequent serogroup was Autumnalis (18/18, 100%) followed by Canicola (12/18, 67%) and Icterohaemorrhagiae (10/18, 56%). In 12 samples, Autumnalis was the serogroup with the highest titers, 1/200 to 1/800. The PCR technique detected the presence of the *rrs* gene (16S) in 10 of 25 (40%) serum samples. The exposure of these asymptomatic animals to leptospires is demonstrated, considering them as potential reservoirs and legitimizing the wide spread of this zoonosis in nature. Leptospirosis studies in Aguará Guazú continue to be a challenge to know the serotypes circulating among these animals in our region and understand their role in the maintenance and transmission of the disease.

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INSECT VECTORS OF PLANT DISEASES

Molting regulative neuropeptides in *Dalbulus maidis,* the corn leafhopper

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Insect growth involves a series of molts during which the old cuticle is shed while a new bigger cuticle is formed. Failure in molting usually results in mortality, making this process an excellent target in the search for new insect pest management strategies. Molting is regulated by enzymes and hormones from different origins, including Bursicon-α (Burs-α) and Ecdysis Triggering hormone (ETH). This work aimed to identify and characterize the function of Burs-α and ETH in the leafhopper *Dalbulus maidis*, an important corn pest in the Americas. We identified the transcripts coding for these two hormones in a transcriptome generated in our lab. To evaluate their physiological relevance, RNA interference experiments were performed. Double-stranded RNA (dsRNA) for each target gene was diluted to a specific concentration (1 µg/µl), and 0.25 µl were injected into 5th instar nymphs. The reduction of Burs-α expression resulted in a lethal phenotype because of the inability of newly emerged insects to expand their wings and defects in cuticle sclerotization. The insects injected with dsETH failed to shed the old cuticle and died. Only a few insects survived, although they could barely come out of the cuticle, which was still attached to them. Surviving insects were able to move, but they could not fly or jump and finally died. In conclusion, the reduced expression of both genes resulted in significant molting impairment and mortality compared to the control group (dsβlac). These results show that RNA interference is a promising new tool for the control of *D. maidis*.

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ARTHROPOD-BORNE DISEASES OF VETERINARY IMPORTANCE

Detection by PCR of *Culicoides insignis* Lutz (Diptera: Ceratopogonidae), the main vector of bluetongue virus (BTV) in the Neotropical region

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Bluetongue Virus (BTV) cause a viral, non contagiosus disease that mainly affects sheep, cattle and wild and farmed ruminants causing damage to these animals and significant economic losses. It is well known in Central America and the Caribbean, and even in the Lesser Antilles, and in South America this virus has been isolated in Brazil, Argentina, Peru, Ecuador and Guyana. *Culicoides insignis* Lutz, the major BTV vector in South America, is one of the most frequent and abundant species found in Southeastern USA, the Caribbean Basin, and Central and South America, that is primarily associated with cattle farms. Accurate identification of biting midges is essential for the understanding of disease epidemiology and vector control. Morphologically, *Culicoides insignis* is placed in the *Culicoides guttatus* group, the females are easily recognized from congeners of this species group by a combination of three wing characters: the r-m crossvein is distinctly dark, the vein R_3 is dark up to the point where it turns abruptly forward to meet the costa, and by the single distal pale spot in cell M_1; other useful characters are the third palpal segment bearing an irregular sensory pit and the distal sensilla coeloconica always present on flagellomeres 1, 3, 5, 7 and 9-13, and sometimes present on flagellomeres 2, 4, 6 and 8. The male wing frequently exhibits a second pale spot at wing margin in cell M_1. In the other hand, the molecular tools applied to taxonomy provide rapid and efficient method to the identification of vector species. We designed a forward primer since a specific sequence of the ITS-1 region of *C. insignis*, which does not generate amplification products in the other analyzed *Culicoides* species. To test the specificity of the primer in vitro we worked with the five most-abundant species captured during the fieldwork in Misiones province. Later, because of the lack of ITS-1 sequences in the species of the guttatus group or some sequence of any neotropical *Culicoides* in the database, the primer in sílico specificity was checked through alignment with other *Culicoides* sequences published in GenBank with the aid of the online BLAST tool, where the alignment was carried out according to the parameters established by default of the program. The use of molecular biology tools applied to the specific identification of species can simplify the taxonomic identification process of *Culicoides* midges and will contribute significantly in the case of cryptic species. With a specificity of 100%, this method could also be used for larval identification and epidemiological surveillance of these species.

Preference of substrate for oviposition of *Mansonia* Blanchard (Culicidae, Mansoniini) from Porto Velho, State of Rondônia, Brazil

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Mosquitoes of the genus *Mansonia* are aggressive and potential vectors of diseases. Immature forms extract oxygen from aquatic macrophytes, common in lentic ecosystems such as dams and lakes. This biological characteristic makes its maintenance in the laboratory challenging. The present study aimed to evaluate the oviposition preference of *Mansonia* in different species of macrophytes, and in polystyrene sheets. Four species were studied; *Mansonia humeralis, Ma. amazonensis, Ma. titillans* and *Ma. indubitans*. The specimens were obtained in the community of São Domingo, located in Porto Velho, Rondônia. In the laboratory, the females were engorged with bird blood and induced for oviposition separately in plastic containers (50 ml) with distilled water. One female of each species was placed on one of the substrates: *Salvinia auriculata*, *Pistia stratiotes*, *Eichhornia crassipes* and polystyrene sheets (~ 0.5 cm thick). The experiments were repeated ten times, and the number of eggs was counted, between 96 and 120 hours later. The non-parametric Kruskal-Wallis test was estimated to verify if the substrates presented statistical differences. Only *Salvinia auriculata* (1,199 eggs) and polystyrene sheet (2,021 eggs) presented eggs. *Mansonia amazonensis* obtained the highest number of eggs in the insectary (n= 1,147). The two substrates did not show significant differences in the amount of eggs (K-W= 2.52, p = 0.11). Our data suggest that *Salvinia auriculata* was the preferred substrate for *Mansonia* oviposition. In addition, the polystyrene sheets did not show significant differences with *Salvinia auriculata*, being an alternative for obtaining eggs of these species in the insectary.

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Zoonotic dirofilariosis in a riparian population of the Río de La Plata

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Cardiopulmonary dirofilariosis (heartworm disease) is a cosmopolitan, serious and potentially fatal disease caused by *Dirofilaria immitis* that mainly affects dogs, while human pulmonary dirofilariosis is a disease that results in pulmonary nodules. Because animal and human dirofilariosis needs the vector activity of culicid mosquitoes for its transmission, adequate environmental factors (moderate/high temperature and humidity) are necessary for both mosquito breeding and the development of infective larvae (L3). *Acanthocheilonema reconditum* is a parasite of zoonotic importance and worldwide distribution. Microfilaremia in canines is mostly associated with the presence of *D. immitis* in the blood and rarely with *A. reconditum*. The life cycle of *A. reconditum* involves an intermediate host, usually fleas or lice. Adults of this species are located in subcutaneous tissue and body cavities where they form nodules. The objective of this study was to determine the prevalence of canine filariosis and seroprevalence in humans living in the neighborhood "El Molino", Punta Lara, Ensenada, Buenos Aires, Argentina. Blood samples from the dogs were analyzed by the clinic immunochromatographic URANO VET® test, specifically detecting circulating *D. immitis* adult antigens and by a modified Knott technique to detect microfilariae (mf). Human samples were analyzed by two laboratory ELISA tests detecting specific anti-*D. immitis* and anti-WSP (*Wolbachia*) IgG antibodies, respectively. A total of 1399 dogs were tested by Knott's test, 117 (8.36%) were positive for microfilariae of a canine filarial species (*Dirofilaria* spp. or *Acantacheilonema* sp.). Of the total number of samples (1399), 580 were tested by URANO VET®, of which 17 were positive (2.1%), and 14 also contained mf. Among the 527 URANO VET® negative dogs, 90 (17%) contained also mf. In addition, 5 out of the 79 human samples analyzed (6.3%) were positive to both antibody tests against *D. immitis* and WSP. These data reveal the complex situation of the dirofilariosis in Ensenada. The existence of canine microfilaremic infections by *D. immitis* is revealed by the simultaneous positivity to URANO VET® and the Knott test. Additionally, the absence of mf in a dog positive for the URANO VET® test reveals the presence of some *D. immitis* amicrofilaremic infections. The existence of numerous microfilarmic samples among dogs with negative URANO VET® test suggests the existence of canine filarial species other than *D. immitis*. Similarly, although human infections attributable to *D. immitis* are detected, we can not rule out the existence of zoonotic infections caused by other canine filarial species, such as *Acantacheilonema* sp. More studies are required involving other techniques that allow the unambiguous identification of the mf in each case.

Molecular detection of *Bartonella* in lice (Phthiraptera: Anoplura) of *Rattus rattus* from Chile

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Bartonella species are facultative intracellular bacteria with zoonotic potential. *Bartonella* can be transmitted by arthropod vectors or through direct contact with infected hosts. Among the mammalian hosts associated with *Bartonella*, rodents are the most speciose group and harbor the highest diversity of ectoparasites with vector potential. Humans are also exposed to this bacterium, since human-rodent interactions are common. In fact, one species, *Rattus rattus* thrives easily in human settlements. *Bartonella* species are transmitted mainly by hematophagous arthropods, and studies have associated lice as an infectious vector of human specific *Bartonella quintana*. Although, fleas are considered competent vectors of *Bartonella*, lice could be important reservoirs of these bacteria. To ensure an adequate control of *Bartonella*, it is necessary to understand the ecology of these bacteria. This study evaluated the presence of *Bartonella* spp. in lice from *R. rattus* in Chile. To this end, lice (*Poliplax spinulosa*) of 286 *R. rattus* from 14 localities (-20.2167S to -53.1667W) were analyzed. DNA extraction was performed from lice pools of 2 to 10 individuals of each host (116 pools). PCRs targetting *Bartonella gltA* gene were performed. The obtained sequences were aligned and compared with the available *Bartonella* sequences in GenBank. A Bayesian inferred tree was constructed in CIPRES (https://www.phylo.org/) using MrBayes (3.2.7a) tool. 3,4% of the pools were positive for *Bartonella* DNA. The phylogenetic reconstruction showed that our sequences were related to *Bartonella coopersplainsensis,* previously detected in *Rattus leucopus* blood from Australia and *R. rattus* from New Zealand. Although, the pathogenic importance of *B. coopersplainsensis* is still unknown, this study could indicate that the lice incorporate the bartonellae from their hosts or in reverse, that the rodents acquire bacteria from the rats, or both. Further analyses with more sequences are needed to have a better understanding of the ecology of *Bartonella* in Chile.

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Analysis of the ectoparasite community in *Rattus rattus* from Chile

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Rodents are the main reservoirs of zoonotic diseases. Among them, *Rattus rattus* has been historically associated with the emergence and dissemination of important human infectious diseases (e.g. typhus, bubonic plague, leptospirosis, hantavirus, etc.). Most of the zoonotic diseases associated with *Rattus* are transmitted by their vector ectoparasites. Among them, the most prevalent in synanthropic rodents are the fleas, followed by mites, lice, and ticks. The aim of this study was to evaluate the mean abundance (MA), and prevalence (P) of ectoparasites (fleas, mites, lice, and ticks) in *Rattus rattus* populations from different localities of Chile. We collected 375 *R. rattus* from 25 localities of Chile (-20.217°; -53.167°S), ectoparasites were cleared and mounted for its observation in microscope. We collected 1,209 ectoparasites (59% lice, 19% mites, 15% ticks, 8% fleas). Two species of lice were identified: *Polyplax* sp. (MA=0.1-5.8, P=12-73%) and *Hoplopleura* sp.. Mites were identified as *Ornithonyssus* sp. (MA=0-7.7, P=4-70%), *Laelaps* spp. (MA=0-0.3, P=1-20%), and Trombidiformes (MA=0.1-0.3, P=3-14%). Identified ticks belonged mainly to the *Ixodes sigelos* group (MA=0-1.7, P=6-67%); however three larvae of *Ornithodoros* were also collected. We found nine flea species. The most abundant and prevalent species were *Leptopsylla segnis* (MA=0-0.4, P=6-50%), followed by *Nosopsyllus fasciatus* (AM=0-0.5, P=8-33%) and then *Sphinctopsylla ares* (MA=0-1.7, P=0-50%). We observed a negative correlation between latitude and abundance of mites (R^2=-0.26; p<0.001). However, we did not found association between latitude and abundance for ticks (R^2=0.06; p=0.31), lice (R^2=0.006; p=0.92) or fleas (R^2=0.09; p=0.11). Lice were the most abundant and prevalent ectoparasites in *R. rattus* from Chile. As we known, lice could be playing an important role in the transmission of pathogenic bacteria among its host and potentially to humans. Among the collected ectoparasites, *Polyplax* sp., *Leptopsylla segnis, Nosopsyllus fasciatus, Sphinctopsylla ares*, and *Ornithonyssus* sp. have been already associated with the transmission of pathogenic bacteria (e.g. *Bartonella*, and *Rickettsia*) in rodents. Further studies with bacteria associated with lice and other ectoparasites of *R. rattus* are necessary to understand the epidemiology of this community.

Detection of *Rickettsia* spp. in ectoparasites of cricetid rodents from Gran La Plata, Buenos Aires Province, Argentina

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Rickettsia spp. are obligate intracellular Gram-negative bacteria, worldwide distributed. The genus includes more than 20 species, many of them causing a group of diseases in humans and animals known as Rickettsiosis, usually transmitted by arthropod vectors. Although in Argentina Rickettsiosis have a low prevalence, clinical cases have been reported in Gran La Plata area. Rodents are usual hosts of ectoparasites, some of which have been involved in the enzootic life cycle of rickettsial species. The aim of this work was to detect bacteria of the genus *Rickettsia* in fleas, mites and ticks associated with cricetids in Gran La Plata. Rodent samplings were carried out in three localities between 2017-2019 by using Sherman-like traps. Captured rodents were anesthetized with ether and sacrificed by cervical dislocation. Ectoparasites were collected in field by brushing the furs of the rodents and stored in alcohol 96% until further molecular studies. DNA was extracted with phenol-chloroform method from individual ticks and from a subsample of mites organized in pools each one of five specimens. For fleas, individual DNA extraction was carried out with Chelex® after an incision at abdominal level to preserve the exoskeleton for further identification. For taxonomic identification at optic microscope, fleas were cleared in 10% KOH and mounted in Canada Balsam and mites were cleared in lactophenol and mounted in Hoyer's medium. Ticks were identified under stereoscopic binocular microscope. A conventional PCR targeting the *gltA* gene (citrate synthase) was performed to detect *Rickettsia* spp. Rodents were identified as: *Oxymycterus rufus* (n=115)*, Akodon azarae* (n=106), *Oligoryzomys flavescens* (n=57); *Scapteromys aquaticus* (n=33); *O. nigripes* (n=12) and *Deltamys kempi* (n=3). A total of 739 ectoparasites collected were examined for the presence of *Rickettsia*: 87,2% were mites (Mesostigmata), 11% fleas (Siphonaptera) and 1,8% ticks (Ixodida). Mites belonged to the family Laelapidae (n=495) and Macronyssidae (n=150), fleas to Rhopalopsyllidae (n=79) and Stephanocircidae (N=2), and ticks to Ixodidae (n=13). PCR amplification of *gltA* showed that from the 81 analyzed fleas, 15 were positive to the presence of *Rickettsia* (18%) (14 of the genus *Polygenis*, Rhopalopsyllidae, and 1 *Craneopsylla*, Stephanocircidae); 23% of the ticks (3 nymphs of *Ixodes*) were positive, while all mites were negative to the presence of *Rickettsia*. This is the first detection of the genus *Rickettsia* in fleas and ticks of cricetid rodents from Gran La Plata. Prevalence of *Rickettsia* among these ectoparasites suggests that they could have a significant role in the enzootic cycle of these bacteria and their importance may be underestimated. Further studies are needed to the specific identification of *Rickettsia* and to examine vectorial capacity and competence of these parasites.

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Canine visceral leishmaniasis and spread of the primary vector *Lutzomyia longipalpis* (Diptera: Phlebotominae), Chaco, Argentina

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Lutzomyia longipalpis during surveillance studies in Chaco between 2010-2012 in the urban and peri-urban areas of Resistencia (Department San Fernando) and rural areas of Colonia Benítez (Department 1 de Mayo) was recorded. Canine visceral leishmaniasis (CLV) was reported by the Ministry of Public Health in 2013 in the last one in the suburban-rural area: Barrio San Pedro Pescador (BSPP) (27°27′S58°52′W) and Puerto Antequera (27°26′S 58°50′W). In 2021, 68 CLV were reported in the metropolitan area and no vector distribution data was studied, so the likely dispersal of *Lu. longipalpis* was assessed. From January-March 2019 (limited by COVID-19 pandemic) and from March 2021 to April 2022, the seasonal capture of Phlebotominae was carried out in Puerto Antequera, 2,500 meters upstream. Five houses (House 1 to 5) according to the critical epidemiological site selected: House 1, 500m from the BSPP, with *Lu. longipalpis* and House 5, 2500m from House1, with active CLV. Two CDC-type light traps were installed in each dwelling, at home (veranda) and peridomicile (up to 20m from the dwelling, in animal pens) from 6:30 p.m. to 8:00 a.m. for 3 consecutive nights. Climatic variables from the national meteorological service was used. *Nyssomyia neivai* 69% (n:818), *Migonemyia migonei* 30% (n:350) and *Lu. longipalpis* 1% (n:10) were recorded. The highest total abundance was in summer 60% (n:703) with an average of 29ºC/81%RH, followed by spring 37% (n:432), 28ºC/82%RH; and autumn 3% (n:43), 19ºC/78%HR. In winter (17ºC/81%RH), no sandflies were captured. The peridomicile registered the highest abundance with a predominance of *Ny. neivai*, 67% followed by *Mg. migonei*, 29%, and *Lu. longipalpis*, 1%. The domicile had the least abundance for the first two and without *Lu. longipalpis*. This was recorded in House 1, maintaining the character of colonization and without dispersion in the rural peri-urban area of the municipality of Colonia Benítez. CVL remained a case and unreported in this area and the role of possible secondary vectors, such as other routes of canine infection, should be studied.

Monitoring of *Mansonia* (Diptera, Culicidae) using automatic *Skeetervac* SV3100 traps in Porto Velho, State of Rondônia, Brazil

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Mosquito monitoring is critical in designing mosquito control strategies. The data obtained from the monitoring allow us to identify the species that occur in a given location, their peaks of abundance, hematophagic behavior, seasonality and even to infer trends of increase or decline in the mosquito population. Thus, automatic traps that collect mosquitoes uninterruptedly become an important tool for entomological surveillance. In the present study, eight points were monitored with automatic traps model *SkeeterVac* SV3100, with Octenol and Lurex attractants, for 35 months, from July 2019 to April 2022, in Porto Velho, Rondônia, Brazil. The collected mosquitoes were removed from the traps weekly, then time series were generated using ARIMA models. The time series indicated successive reductions in the abundance of Mansonia. In addition, small abundance peaks were also detected after small cycles of deforestation for family farming in places close to the traps. Our results support the use of *Skeetervac* SV3100 traps to estimate trends in the abundance of Mansonia sp. even if the specimens collected in the trap did not present good quality for more specific identifications.

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Epidemiology of the flea *Tunga penetrans* in affected neighborhoods of Puerto Iguazú, Misiones

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For the period 2021-2030, WHO launched an action plan that includes tungiasis as one of the neglected tropical skin diseases. The plan proposes reinforce skills health workers to know and identify the disease, understand the risk factors according to the context and living conditions of the affected communities, and promote the implementation of integrated interventions. This disease is a health problem in the tropics and subtropics, exacerbated by social, economic, political and environmental conditions. *Tunga penetrans*, the causal agent of this zoonotic ectoparisitosis, is known by different names depending on the region: *pique* in Argentina, *bicho do pé* or *pulga da areia* in Brazil, *nigua* in Colombia and Costa Rica. Studies carried out in Brazil show high incidences in humans and domestic animals associated with disadvantaged areas with a high index of social vulnerability and poor sanitary conditions. In our country, cases have been reported in Salta, Jujuy, Tucumán, Santiago del Estero, Santa Fe, Chaco, Formosa, Misiones and Corrientes. This study analyzed the knowledge, attitudes and perceptions of the inhabitants of the neighborhoods of Puerto Iguazú, regarding tungiasis and its association with care and prevention practices, and their effectiveness. Thirty-eight homes were randomly selected from urban and periurban areas, and through the application of semi-structured surveys, different aspects related to the constructed and naturalized knowledge about tungiasis were addressed. The information collected was grouped into five categories: 1) sociodemographic factors, 2) factors associated with housing, 3) presence and habits of domestic animals, 4) knowledge, attitudes and practices related to tungiasis, 5) perceptions of the actions of the Municipality-Ministry of Health. A qualitative analysis of the data obtained in the surveys was carried out and measurements and percentages of the five categories were compared between the two areas sampled. Most of the people interviewed expressed that they knew about the sting, having been affected at least one of their family members, mainly children, by its sting in the last six months. Some of the causal factors for the high prevalence of flea bites were: lack of information on the flea's life cycle, its treatment and prevention; lack of protection due to the absence or type of footwear used; lack of access to products for the control of ectoparasites; negligence of municipal and provincial health and zoonosis services. In this sense, transdisciplinary research and training of health promoters would provide, on the one hand, information on preventive measures, specific use of repellents and insecticides, identification of pique breeding sites and their reservoirs, and on the other hand, health support to the community in the correct treatment, use of instruments, ways of extraction and disinfection to avoid risks of secondary bacterial infections.

Studies of the dinamics of *Anaplasma marginale* strains in the vector and the mammalian host through genotyping

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Anaplasma marginale is a gram-negative obligate intracellular tick-borne bacterium that infects erythrocytes of ruminants and other mammalian host species from tropical and subtropical world regions. In Argentina, the transmission of *A. marginale* is mainly associated to the one-host tick, *Rhipicephalus microplus.* Although the presence of *A. marginale* DNA was previously confirmed in *R. microplus* larvae by PCR, the transovarial transmission of the bacterium in the tick remains neglected. The aim of the present study was to detect and molecularly characterize *A. marginale* in the bovine and in organs from *R. microplus* engorged female removed from the host, in order to determine the *A. marginale* genotypes involved in the transmission process. For this purpose, blood and tick samples were obtained from a bovine with acute Anaplasmosis at an endemic region in Corrientes province, Argentina. In order to get rid of the host blood meal, we dissected a female engorged tick and extracted the ovaries and salivary glands. DNA was extracted from 400 µl of the bovine blood sample using the ADN PuriPrep-S kit (INBIO Highway) and from the tick tissues using the NucleoSpin Tissue kit (Macherey-Nagel). For *A. marginale* identification, we used a species-specific PCR protocol that amplifies two copies of the *msp1β* gene. We also amplified, cloned and then sequenced the 5' end repetitive region of the *msp1α* gene, broadly used for genotyping *A. marginale* strains. We detected *A. marginale* in the bovine blood sample and in the tick organs. We analyzed 30 clones from the bovine blood sample and 8 clones for each tick organ. We then determined the number and type of tandem repeats for each MSP1α coding region. There was a great diversity of genotypes in the bovine (19) that were formed by different combinations of 20 repeats, with a minimum of one (AR12) and a maximum of 8 (AR9-62-61-AR10-62-62-61-AR14). Engorged females organs showed a fewer number of different genotypes (ovaries: 2; salivary glands: 3) and repeats involved (8). Two genotypes found in the engorged female (1 in salivary glands -AR15-62-62-62-61- and other in ovaries –AR9-62-66-62-61-) were not detected in the bovine host which could be the result of a previous transovarial transmission process of *A. marginale*. The high number of *A. marginale* genotypes detected in the infected bovine and the reduce number in the tick organs suggest a diverse genotype fitness at least for transovarial transmission. Moreover, these results help to explain how a monoxenic cycle tick as *R. microplus,* could be responsible for the large distribution of anaplasmosis in enzootic regions.

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Meta-analysis of the microbiota of sandflies from the Old and New World

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Sandflies (Diptera: Psychodidae) are vector insects of different etiological agents responsible for transmitting diseases with great medical and veterinary importance in public health, such as leishmaniasis. However, it is known that insects, in general, have an important interaction with the symbiotic microorganisms that make up their microbiota. The microbiota, in turn, is essential for the development of the host by providing nutrition, digestion and immune responses against pathogens. Although there is knowledge about this interaction, little information is available on the sandfly microbiota. Thus, the present study aims to perform a meta-analysis of the microbiota of *Phlebotomus* species *(Phlebotomus papatasi, P. dubosqui, P. argentipes, P. sergentii, P. kandelakii, P. profileiewi, P. halepensis, P. chinensis, P. perniciosus* and *P. major*) and *Lutzomyia* (*Lutzomyia longipalpis, L. cruzi, L. evansi, L. neivai* and *L. intermedia*) by comparing the dependent and non-dependent techniques of cultivation of the articles using sandflies old and new world vectors. For this, a survey of the analyzes of published studies was compiled that evaluated the bacterial richness through the dependent and independent methods of culture of a set of species of the two genera *Lutzomyia* and *Phlebotomus*. Thus, it was possible to observe a total of 81 bacteria, 50 Gram-negative and 31 Gram-positive bacteria, both found in both genera. So far about 60% of the bacteria have been identified in the New World (*Lutzomyia*) and 40% in the Old World (*Phlebotomus*). Bacillaceae, Enterobacteriaceae, Pseudomonadaceae and Xanthomonadaceae were shared between at least eight species from the Old (*Phlebotomus*) and New World (*Lutzomyia*). While in the New World the following families of bacteria (Actinobacteridae, Alicyclobacillaeae, Bacteoidaceae, Bogoriellaceae, Cellvibrionaceae, Chitinophagaceae, Corynebacteriales, Cytophagaceae, Deferribacteraceae, Desulfovibrionaceae, Erysipellotrichacedae, Glycomycetaceae, Idiomarinaceae, Lachnospiraceae, Leptotrichiaceae, Nitrobacteraceae, Prevotelaceae, Rhodanobacteriaceae, Riconellaceae, , Selenomonadaceae, Shingobacteriaceae, Sporolactobacillaceae, Thermomonosporaceae, Vibrionaceae and Weeksellaceae) were exclusive to *L. longipalpis* and one family (Opitutaceae) to *L. neivai*. In the Old World, nine families of bacteria are exclusive to the following species *P. papatasi* (Alphaproteobacteria, Oxalobacteraceae and Plataspidae), *P. chinensis* (Planctonycetaceae, Rhodospirillaceae and Spiroplasmataceae), *P duboscqi* (Chloroflexaceae), *P. sergentia* (Cellulomonadaceae;) and *P. perniciosus* (Nocardiaceae.). With these results it is possible to better understand the interaction of the microbial community between sand flies, in addition to providing potential target bacteria for future studies of paratransgenesis.

Epidemiological relevance in the detection of *Bartonella henselae* in wild and synanthropic rodents in Argentina

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The genus *Bartonella* comprises gram-negative bacteria associated with emerging diseases in animals and humans, known as bartonellosis. Cat scratch disease (CSD) is the best known bartonellosis in humans, and is caused by *Bartonella henselae* and *Bartonella clarridgeiae*. Rodents are natural reservoirs for many *Bartonella* species, and fleas have a key role in the transmission cycle. The prevalence of *Bartonella* varies according to the environments and hosts involved. Currently, in Argentina, Bartonella infection is known in rodents of the species *S. aquaticus, D. kempi* and *Oligoryzomys flavescens*. In the province of Misiones, a case of Bartonella encephalitis was reported in a young girl who lived in close contact with flea-infested cats. In turn, *B. clarridgeiae* was detected in the blood of domestic cats and in *Ctenocephalides felis felis* fleas. Due to its scarce knowledge, the aim of this study was to detect and molecularly characterize *Bartonella spp.* in rodents from Misiones. DNA was extracted from spleen samples of 123 rodents (55 *Rattus rattus*, 24 *Mus musculus*, 36 *Akodon montensis*, 5 *Calomys tener*, 3 *Oligoryzomys nigripes*) captured in environments with different degrees of anthropogenic disturbance: urban and peri-urban areas, anthropized forest environments, primary and secondary forest areas, and agricultural crops. Nested PCR was performed using Bartonella genus-specific primers that amplified a fragment of the citrate synthase enzyme gene (gltA). PCR products were sequenced and compared with sequences stored in the NIH/NCBI (National Center for Biological Information) database. *Bartonella spp.* were detected in: *R. rattus* 5/55 (9.1%) captured in urban and peri-urban areas of Puerto Iguazú, *A. montensis* 2/36 (5.5%) in peri-urban area and anthropized jungle environments, and *C. tener* 1/5 (20%) in agricultural crops in Wanda. Sequencing and phylogenetic analyses allowed the identification of 3 genotypes in rodents, corresponding to *B. henselae* detected in *R. rattus*, and for the first time worldwide in *A. montesis* and *C. tener*. The presence of *B. henselae* in rodents in anthropized areas has public health implications, given the close interactions between human populations, animals and their fleas, due to the potential epidemiological nexus with cat scratch disease. Our results provide the first evidence of Bartonella in wild and synanthropic rodents, evidencing the need for further studies for the development of specific monitoring measures to prevent the emergence of zoonotic diseases in interface areas of Argentina.

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Susceptibility of *Mansonia* Blanchard (Culicidae, Mansoniini) to *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* in Porto Velho, State of Rondônia, Brazil

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Biolarvicides are agents that stand out worldwide in the control of medically important mosquitoes, reinforcing the initiative to control without harm, and avoiding pressure for resistance in other species. Given this versatility, we evaluated the susceptibility of two products, Vectobac-WG (*Bacillus thuringiensis* var. *israelensis*-Bti) and Vectomax-FG (Bti and *Bacillus sphaericus*-Bsp), against 1st instar larvae (L1) of *Mansonia humeralis* and *Ma. titillans*. To obtain the larvae, adult females were captured and engorged in the field, in the district of Jaci-Paraná, Porto Velho, Rondônia. The assays followed the World Health Organization (WHO) protocol, in an environment with controlled temperature (T) and relative humidity (RH) (T = 25-27ºC / RH - 70-80%). Six concentrations were used in triplicates, with 4 replications, including 'treatment' and 'control'. Each test was performed with ~50 larvae (L1) in a container (500ml), containing distilled water and a plant as substrate. Concentrations were estimated for each product (ppm). The non-parametric Kruskal-Wallis test was calculated for multiple comparisons between the concentrations tested. After the end of the tests, it was observed that the two species of *Mansonia* are highly susceptible to the biolarvicides analyzed. There were no significant differences between two species of mosquitoes. However, mortality rates were differences between concentrations. The highest concentrations reached 100% mortality in both products, and the lowest reached a mortality rate ≤ 54%. The tests carried out showed high effectiveness for the two biolarvicides against *Mansonia* larvae (L1) in the laboratory, therefore their use in the field is promising.

Culicoides (Diptera: Ceratopogonidae) in the state of Sao Paulo: morphological identification, DNA barcoding and molecular detection of pathogens

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Culicoides Latreille biting midges (Diptera: Ceratopogonidae) are dipteran insects with 151 species in Brazil. Females of *Culicoides* spp have hematophagous habits and can act as biological vectors of several pathogens that cause diseases in humans and animals. However, studies on the distribution of *Culicoides* species and presence of arboviruses, *Haemoproteus* (*Parahaemoproteus*) sp and filariae in Brazil and, mainly, in the state of São Paulo are scarce. Here, we aim to expand the species described in the state and identify potential vector species of pathogens. The collections took place using CDC traps in two different biomes: Savanna and Atlantic forest (plateau and coast). The morphological identifications were carried out using images constructed in layers through a high definition stereomicroscope and specific taxonomic keys, in addition to a comparison with the Ceratopogonidae Collection (Fiocruz/CCER). Presence of flavivirus and chikungunya virus was tested by qPCR, while *Haemoproteus* was screened by nested PCR. Also, sequencing of the *Culicoides* DNA barcoding region was performed and a phylogenetic tree was generated adding reference sequences found in the Genbank® database. The results obtained so far showed three species morphologically identified, all of them with previous records in the state. At the São Paulo Zoo Foundation, located in the state capital, 137 female *Culicoides* were collected and ten individuals photographed were identified as *Culicoides* (*Psychophaena*) *venezuelensis.* In Santa Rita do Passa Quatro, a municipality in the northeast of the state, 200 individuals were collected, 25 of which were morphologically identified as *Culicoides* (*Haematomyidium*) *debilipalpis*. In São Sebastião, a municipality on the northern coast of the state, 174 individuals were collected, with 12 being morphologically identified as *Culicoides* (*Hoffmania*) sp (*guttatus* group). A total of 33 individuals from the São Paulo Zoo Foundation were tested by qPCR for arboviruses (flavivirus and chikungunya), with negative results. All 47 morphologically identified specimens were negative for *Haemoproteus* by nested PCR. Finally, analyses of partial mitochondrial cytochrome oxidase subunit I gene (COI) sequences were used to infer phylogenetic relationships among the specimens of the three identified species and sequences from the species available in the GenBank. To our knowledge, this is the first study investigating *Culicoides* spp. in the São Paulo Zoo Foundation, the biggest of Brazil. Thus, this research expanded the knowledge about the distribution of *Culicoides* species in the state of São Paulo, in addition to contributing with new sequence data for Brazilian species of this insect group.

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In-vitro tests for the biocontrol of *Rhipicephalus microplus* with entomopathogenic fungi in Uruguay

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The common cattle tick, *Rhipicephalus microplus*, generates millionaire losses in the livestock industry worldwide (US$18 billion/year). In addition to its direct pathogenic effects, as anemia and low production, it´s also the vector of *Babesia bigemina, B.bovis* and *Anaplasma marginale*, agents that cause babesiosis and anaplasmosis, which often lead to the death of animals. Control with chemical products has been difficult in recent decades due to the development of resistance, residues in animal products and subproducts and environmental contamination. In Uruguay, acaricide resistance is widely dispersed, and multi-resistant populations have been diagnosed complicating its control, therefore, alternative methods are being sought. One alternative is the use of entomopathogenic fungi as biological controllers. Prior to *in-vivo* trials, *in-vitro* efficacy bioassays should be performed. The objectives of this work were to study the *in-vitro* efficacy of four strains of entomopathogenic fungi so they can be used as controllers against *R. microplus*. The strains selected were two of *Metarhizium anisopliae* (Ma2411, Ma2118) and two of *Beauveria bassiana* (BG, Bb2121) belonging to the fungal collection of the University of the Republic. Pure cultures were performed and replicated to prepare final suspensions diluted in 0,02% Tween 80 at a concentration of 1x10^{8}spores/ml. Viability and germination were assessed in potato dextrose agar (PDA) plates. Engorged female ticks obtained from susceptible strain (Mozo) of *R. microplus* were used. Dead or non-viable ticks were discarded. Healthy ticks were disinfected with 0.3% chlorinated water and distributed in homogeneous groups of 10 ticks, in terms of weight and size. The treatments were applied by immersion in 20ml of suspension for one minute, then were incubated at 27ºC and 80% humidity. The control group was immersed in 0,02% Tween 80. All treatments were performed in triplicate. On days 7 and 14 the egg mass was weighed and on day 50 larval hatching was evaluated. Reproductive efficiency (RE, calculated as RE = (gr of eggs / gr of *teleogins*) * % larval hatch) of the treatment and control groups was calculated and subsequently the percentage of efficiency (%Ef={(RE control - RE treated) / RE control} x 100). With the results obtained so far (%Ef Ma2411=28; %Ef Ma2118=13; %Ef BG=8,5; %Ef Bb2121=22), we can suggest that although the use of this fungal strains was not highly efficient for the control of *R. microplus* when applied individually, they can nevertheless be part of an integrated pest management plan. Although these organisms cannot be considered for an eradication plan for *R. microplus*, they can be effective biocontrollers if applied in combination with other control strategies. To increase the efficacy of these strains, simultaneous use with different acaricides is proposed. Even though these preliminary results are promising, further studies are required.

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VECTOR RESISTANCE TO PESTICIDES USED IN PUBLIC HEALTH

Aedes aegypti intestinal microbiota and the relationship with the susceptibility level to temephos and permethrin insecticides

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The constant use of insecticides such as organophosphates and pyrethroids, for the control of the *Ae. aegypti* has led to the development of resistance to them. Therefore, in the present study, the level of susceptibility to the insecticides temephos and cis-permethrin in the population of Guadalupe, N.L. was determined by means of the bioassay methodology proposed by the World Health Organization (WHO, 2016). Likewise, the samples were characterized to determine the susceptibility. The samples were collected in the town of Guadalupe, Nuevo León, Mexico, they were taken to the medical and veterinary entomology laboratory of the UANL, for the breeding of the generation, once the F1 generation was obtained, the susceptibility tests were carried out (WHO, 2016), subsequently the CL_{50}, CL_{99} and DD were determined. The aforementioned test was replicated with the diagnostic dose, after 24 hours, the live and dead larvae were selected, placed in lysis tubes, after which the extraction was performed with the DNA ZymoBiomicsTM kit, then the PCRs were performed with the Quick-16STM NGS kit, an Ilumina equipment was used to determine the sequencing results, and the DADA2, Qime2 and R programs, finally used the alpha and beta diversity indices to determine the diversity of the samples. The results obtained showed that the population of Guadalupe presents a CL_{50}: 0.022 ppm for temephos, for cis-permethrin a CL_{50}: 0.071 ppm. In the susceptible population New Orleans (NO), an CL_{50}: 0.003 ppm for temephos and in cis-permethrin an CL_{50}: 0.001 ppm were obtained. In relation to the microbial communities at the phylum level, they indicate that Proteobacteria is the one with the highest abundance with 66.4%, Bacteroidetes with 19.8%, Actinobacteria with 11.4% and the rest represents less than 5% of the abundance of the 6 treatments. At the species level, there were no bacteria for both live and dead treatments of both insecticides, which could influence greater or lesser susceptibility, there were differences in the number of species, with the field being the one with the greatest diversity with 301, being *Rhodocyclaeae_Na_sp* with 31.93% the most abundant, the New Orleans treatment was the least diverse with 36, *Elizabethkingia meningoseptica* being the most abundant with 38.12%.The alpha diversity indices, such as the Shannon, Chao1 and Fisher indices, indicate that the treatment with the most diversity is field, while for Simpson, it was the live cis-permethrin treatment that presented the greatest diversity, finally, on the part of the beta index, Bray Curtis, there was similarity between the living and dead treatments of temephos, on the other hand, for both living and dead cis-permethrin, dissimilarity was presented. In conclusion, the intestinal microbiota in larvae of *Ae. aegypti* does not influence a lower or higher susceptibility to insecticides.

Kdr mutations associated with pyrethroid resistance in *Aedes aegypti* (Diptera: Culicidae) mosquitoes from Argentina

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The *Aedes aegypti* mosquito possess sanitary relevance as a vector of the dengue, chikungunya, zika and yellow fever viruses, which cause thousands of deaths per year in the world. During epidemic periods, dengue control strategies include the use of pyrethroid insecticides. Pyrethroids cause insect death by modifying the normal function of proteic voltage-gated sodium channels (Nav) in the membrane of excitable cells. Specific point mutations of the Nav genes, called kdr mutations, are a major cause of pyrethroid resistance in insects. Increase in the frequency of two kdr mutations called V1016I and F1534C have been reported in American continent natural populations of *Ae. aegypti.* Despite this, the presence, abundance and distribution of these mutations have not been reported to date in Argentina. This work proposes the identification, monitoring and study of the distribution of kdr mutations in populations of Ae. aegypti from Argentina. For this, a technique developed in our laboratory called High Resolution Melting (HRM) based on quantitative real-time PCR (qRT-PCR), has been implemented. We found the presence of both V1016I and F1534C mutations in Tartagal (Salta Province), whereas in the Metropolitan Area of Buenos Aires only F1534C was detected. This is the first report on kdr mutations in Argentina. Resistance monitoring is a fundamental requirement for prolonging the life span of insecticides and the planning of viable control alternatives.

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Comparison of intensity resistance rapid diagnostic test using impregnated paper and bottle impregnated in *Aedes aegypti* populations in Brazil

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The principal mosquito vector of certain arboviruses in the Americas, *Aedes aegypti*, has been a focus for diseases management. The primary method of vector control is the use of insecticides, which results in the selection of mosquitos resistant to these chemicals. The evaluation of insecticide susceptibility in Ae. aegypti populations is a critical component of vector control efforts. Adult mosquito resistance is widely assessed using insecticide-impregnated papers (WHO) and insecticide-impregnated bottles (CDC). Each laboratory uses the approach that best fits your reality. Brazilian populations were evaluated for resistance to the insecticide deltamethrin using paper and bottle methods, and the correlation between the results obtained was verified. Eggs from 18 populations were collected throughout Brazil as part of the national insecticide resistance monitoring program for Ae. aegypti between 2017 and 2018. Three to five days old females (F1 or F2 generation) were submitted to the Intensity of Resistance Rapid Diagnostic Test of (I-RDT) in the WHO deltamethrin impregnated papers method, with doses equal to 1 x, 5 x and 10 x the diagnostic dose (DD) (0.03%, 0.15% and 0.3%) and in the CDC deltamethrin impregnated bottles method, with doses equal to 1 x, 5 x and 10 x the diagnostic dose (10 µg/ml, 50 µg/ml and 100 µg/ml). In the impregnated paper bioassay, no population was susceptible at the 1 x DD or at the 5 x DD. At the 10 x DD, six populations showed mortality above 98%, indicating moderate resistance. The remaining 12 populations showed mortality below 98%, indicating high-intensity resistance to deltamethrin. In the bottle bioassay, no population was susceptible at the 1 x DD dose. At the 5x DD dose, nine populations showed mortality above 98% and were classified as having low intensity of resistance. At the 10 x DD dose of the nine populations evaluated, seven showed mortalities above 98% and were classified as having moderate intensity of resistance, and two populations showed mortality below 98% and were classified as having high intensity of resistance. The two methodologies presented the resistance intensity differently for nine populations at the 5xDD dose and comparable results at the 10xDD dose for just three populations. Following the interpretation of Cohen (1988), a strong correlation was verified between the findings obtained at the intermediate dose of the two methods ($r=0.53$; $P=0.0244$), but no correlation was found between the other doses evaluated. In a qualitative conclusion, this study highlights that the WHO and CDC bioassays produce similar results regarding mosquito susceptibility to the insecticide deltamethrin (resistant or susceptible), however the methodologies may produce different findings for the same population in terms of intensity strength of resistance.

Insecticide resistance associated to environment in *Triatoma infestans*: what do we know and what remains unknown

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Resistance to insecticides is considered as a barrier to chemical control of *Triatoma infestans*, the main vector of Chagas disease in the Southern Cone of South America. Although initiatives of disease incidence reduction in the area have integrated different strategies, they have been based mainly on vector elimination through pyrethroids insecticides like deltamethrin. The first reports of pyrethroids resistance were found in *T. infestans* populations from Salvador Mazza (northern Argentina) and Yacuiba (southern Bolivia). Recently, a mosaic pyrethroid resistant focus was described in the center of the Argentine Gran Chaco (General Güemes department, Chaco province), characterized by the presence in the same area of susceptible and very high resistant populations. Different resistance mechanisms were proposed to be involved together with the contribution of environmental variables that promote the toxicological heterogeneity described. In this context, new questions arise: Is there another resistant focus along *T. infestans* Argentine endemic zone? Are the environmental predictors influencing the distribution of resistance at endemic area scale? To contribute to this knowledge, we studied toxicological information from insects collected and analyzed in 224 Argentine localities during 2010- 2020 as part of the resistance monitoring performed by Chagas National Programme. The sites were classified according to insects survival rate exposed to deltamethrin discriminant dose: with 0- 0.19 were considered susceptible, 0.2-0.79 low resistant, and 0.8- 1 high resistant. Then, it was georeferenced in order to describe the spatial distribution of resistance and to set up spatial variables (demographic, land use, urbanization, connectivity, climatic) potentially related to resistance. The association between resistance and spatial variables was studied using generalized linear models (GLM), with error distribution selected according to the response variable definition: the number of surviving insects was modeled with Poisson error distribution with log link, while the presence/ absence of resistance was modeled with Binomial error structure with $n= 1$ (Bernoulli distribution) and logit link function. Concerning spatial distribution, 197 susceptible localities were spread along the endemic zone. Resistant localities with different survival rates were registered throughout the area, 9 of them with high resistance circumscribed to the two resistant foci described by the moment. 18 localities with low resistance were present in Chaco, Salta, Tucumán, Catamarca, Formosa and Santiago del Estero provinces, highlighting their relevance in control activities planning. Precipitation variables were associated with resistance in all GLM evaluated. Models of presence/absence were the most accurate, with precipitation, distance to the capital city and land use contributing to resistance distribution. This information could be valuable to improve control strategies of *T. infestans* in future scenarios characterized by unpredictable changes in land use and precipitation.

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Wikimedia: a toolbox for the democratization of the scientific knowledge associated with vector ecology. A view from the Global South

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Wikipedia, the free, multilingual, online encyclopedia, is currently the most consulted source of knowledge in human history. It belongs to a movement for free information and has the goal of making knowledge created by humankind available. Wikipedia is part of a bigger project called Wikimedia, which includes a multimedia repository (Wikimedia Commons), and a database that links the different projects (Wikidata), along with other less known initiatives. A characteristic of Wikimedia is that it can only include previously published knowledge (it cannot be a primary source) and its content is created and improved constantly and collaboratively by thousands of volunteers. Virtually any person can edit it if they follow some simple editorial rules. Even so there is a huge gap and some significant biases in the contents of Wikipedia, and several extensive fields are underrepresented. From the Equipo de Wikimedistas del Museo de La Plata [Wikimedians' Team of the La Plata Museum] (Argentina) – an interdisciplinary group of students, scientists, and professionals from different fields – we address this lack of knowledge training new users, spreading information about Wikimedia, and organizing campaigns of Wikipedia edition ("editathons") in Spanish that are focused on specific topics with a regional perspective. Nowadays the impact of disclosure in Wikipedia surpasses even the wildest imagination. Every month this encyclopedia is consulted over 2 billion times, and if we only consider articles edited by members of our team from May 2020 to date, they have reached over 150.000.000 views. Here we show the result of our latest editathon which focused on "Biological Vectors". The first step when organizing this editathon was to improve the article Vector biológico [Biological vector], which was confusing and incomplete, and which ended up being combined with the article Vector epidemiológico [Epidemiological vector]. We organized the edition into three main topics: (1) Vectors that affect humans, (2) Vectors of veterinary importance, and (3) Vectors with phytosanitary importance. The editathon had 18 participants, 7 of whom were entirely new users. During the event a total of 104 pieces of content were either improved or created on Wikimedia. We identified 89 taxa that are known biological vectors with a specific tag that allows an easy search of vector organisms within Wikipedia. We created or improved 58 articles related to vector taxa and vector-borne diseases on Wikipedia, and uploaded 6 new figures of vector organisms on Wikimedia Commons. We created or improved a total of 40 entries on Wikidata. Finally, we greatly improved the existing article about Chagas' disease. This activity has made valuable information accessible to the community regarding biological vectors and the diseases they transmit.

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