Biodiversity and public health interface

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Abstract: Alongside modernity, the human activity has been a key factor in global environmental risks, with worldwide anthropic modification being the cause of the emergence of diseases for wild and livestock animals, and even humans. In special, the increase in the spatial distribution and in the incidence of some emerging infectious diseases (EID) are directly associated to deforestation and global climate changes. Moreover, the arise of new EID agents, such as the SARS-COV-2 have been reported for the last 30 years. On the other hand, biodiversity has been shown to be a key indicator for ecosystem health, and to pose a role to increase the promotion of human public health. In neotropical regions, and in special, in Brazil, several infectious diseases have been demonstrated to be directly affected for the biodiversity loss, such as malaria, hantavirus pulmonary syndrome, yellow fever, urban arboviruses, spotted fever, amongst other. To better understand the ecosystem capacity of regulation of infectious diseases, FAPESP BIOTA program have supported researchers and research projects to increase knowledge about Brazilian biodiversity and the ecosystems, such as diversity of bird bioagents, venomous animals biodiversity, diversity of mosquitos species in forest patches inside urban areas, propagation of the yellow fever virus over fragmented forest territories, loss of ecological corridors and occurrence of spotted fever and malaria, amongst others. It is noteworthy that FAPESP BIOTA is a successful program and must be expanded as an important tool for present and future public health promotion.

Keywords: One health; Emerging infectious diseases; Zoonoses regulation.

Biodiversidade e a interface com a saúde pública

Resumo: Junto à modernidade, a atividade humana tem sido um fator chave ligada aos riscos ambientais globais, as modificações antrópicas em âmbito mundial têm sido causa do surgimento de doenças para os animais silvestres e domésticos, bem como para o ser humano. Em especial, o incremento na distribuição espacial e incidência de doenças infecciosas emergentes (DIE) estão diretamente associados ao desmatamento e às mudanças climáticas globais, além disso, o surgimento de novos agentes de DIE, como o SARS-COV-2, tem sido relatado nos últimos 30 anos. Por outro lado, a biodiversidade tem se mostrado um indicador chave para a saúde dos ecossistemas, além de representar um papel importante na promoção da saúde pública humana. Nas regiões neotropicais, e em especial, no Brasil, várias doenças infecciosas têm demonstrado ser diretamente afetadas pela perda de biodiversidade, como a malária, a síndrome pulmonar por hantavírus, a febre amarela, as arboviroses urbanas, a febre maculosa, entre outras. Para entender melhor a capacidade ecosistêmica de regulação de doenças infecciosas, o programa BIOTA FAPESP tem apoiado pesquisadores e projetos de pesquisa para aumentar o conhecimento sobre a biodiversidade e os ecossistemas brasileiros, como a diversidade de bioagentes de aves, a biodiversidade de animais peçonhentos, a diversidade de espécies de mosquitos em fragmentos florestais dentro de áreas urbanas, a propagação do vírus da febre amarela em território florestal fragmentado, perda e isolamento de remanescentes florestais e a ocorrência de febre maculosa e malária, entre outros. Ressalta-se que o BIOTA FAPESP é um programa de sucesso e deve ser ampliado como importante ferramenta de promoção da saúde pública presente e futura.

Palavras-chave: Saúde única; Doenças infecciosas emergentes; Regulação de zoonoses.
Introduction

In 1992, the concept of “risk society” was proposed to describe one of the central problems in modernity, a global environment where the risks to society are spread worldwide and they are dynamic and heavily influenced by or created directly through human activity (Beck 1992). In particular, this scenario fits a potential increase in risks associated with zoonotic diseases.

Numerous emerging infectious diseases (EIDs) have arisen or been detected in wild animals, noteworthy, EIDs present important health implications for human beings, livestock, and wildlife. In late 2019 the virus SARS-CoV-2 arouse in the city of Wuhan in China and spread worldwide, later on it was demonstrated that the virus is closely related to bat-borne coronavirus, SARS-CoV-2 adapted to infect human beings, through the phenomenon known as spillover (Zhou & Shi 2021), when etiological agents, once restricted to wildlife or livestock, adapt and begin infection process and sustained transmission on human populations.

Humans also may play this role, and infect other animals, this phenomenon is known as spilloback and was reported during the Covid19 pandemic, such as in Netherlands, where an outbreak of SARS-CoV-2 was detected in a mink farm (Oude-Munnink et al. 2021), also domestic cats and dogs, as well as tigers in zoos, have also been found infected (Hu et al. 2020, McAloose et al. 2020). Spillback also was reported in wildlife, in North America, a population of free-ranging white-tailed deer (Odocoileus virginianus) was detected infected with SARS-CoV-2, moreover, the virus was capable to be sustaining transmitted among the wild individuals, with important mutation in the genetic sequence that transcribes the virus spike protein, which were not seen in human samples, this interaction opens new pathways for viral evolution and rises a potential threat for public health in future (Hale et al. 2021).

Besides health and environmental threat, EIDs also pose a significant potential issue to global economy and global security (Heymann et al. 2015, Morens & Fauci 2012), as it has been observed significant potential issue to global economy and global security (Heymann et al. 2015, Morens & Fauci 2012), when etiological agents, once restricted to wildlife or livestock, adapt and begin infection process and sustained transmission on human populations.

The research in the area of knowledge developed under BIOTA.

Malaria

The dynamics of malaria transmission was studied in depth in the project “Malaria transmission dynamics at different thresholds of landscape fragmentation (2014/09774-1)” by the researcher Gabriel Zorello Laporta (FUABC). Malaria is an endemic transmissible disease in the Amazon, with an average of ~222,000 cases reported every year (Prist and Laporta, In Prep). *Plasmodium vivax* and *Plasmodium falciparum* are the most prevalent malarial parasites, and *Anopheles darlingi* is the main vector. Vector-borne diseases, i.e., pathogens transmitted by biting arthropods, are particularly sensitive to forest loss and fragmentation through changes in host and vector communities, vector breeding habitat, microclimate suitability for pathogen development, and vector–human contact rates, among others (Vittor et al. 2006, 2009).

In this FAPESP project, Laporta et al. identified determinants tied with the emergence of malaria incidence risk in the Amazon basin, and that the pattern of deforestation level (spatial effect) and the process of deforestation timeline (temporal effect) are indissociable determinants of malaria risk. Not only forest loss, but also the age of deforestation is an important factor in determining transmission risk, exiting an association between malaria risk, population and deforestation in recently colonized settlements. The greatest malaria risk occurs when accumulated deforestation is higher than 50% in the first 25 years of settlement colonization—new and degraded settlements encompass the greatest risks (Laporta et al. 2021). Pioneers inhabit poor housing on the forest edges in these recently deforested settlements. Settlers usually store ground waters that are employed to prepare meals, wash clothes, and take baths. In these waters, the principal vector, *An. darlingi*, breeds as immatures until the completion of their development to the adult stages. These anophelines seek blood in the houses nearby and, if any settler is already Plasmodium-infected, transmission cycle of malaria successfully starts (Oliveira et al. 2021). Composition and configuration of the human-modified natural landscapes that favor malaria risk was also estimated (Chaves et al. 2021): landscapes having intermediate forest cover (~70–30%) and higher levels of forest fragmentation have the highest risk of malaria transmission risk to humans. This happens because of intensification of the contact between humans and *An. darlingi*, with forest cover loss leading to a decrease in mosquito species diversity and increasing the abundance of this malaria vector (Chaves et al. 2021).
The transmission of autochthonous malaria outside of the Amazon region occurs in several Brazilian states, mainly those associated with the ecosystem of Atlantic Forest in Southeastern and Southern Brazil (de Pina-Costa et al. 2014). Evidence suggests that malaria is a zoonosis in these areas as human infections by simian Plasmodium species have been detected, and the main vector of malaria, *Anopheles (Kerteszia) cruzii*, can blood feed on human and simian hosts.

A new mathematical model to explain the dynamics of zoonotic transmission of malaria in these areas was proposed (Medeiros-Sousa et al. 2021) during the project “Mosquito Biodiversity (Diptera: Culicidae) in the Cantareira State Park and in the Environmental Protection Area Capivari-Monos, State of São Paulo” (2014/50444-5) coordinated by Mauro Toledo Marrelli, from the School of Public Health of the University of São Paulo (FSP/USP-SP). Simulations performed with the model indicate that the dynamics of zoonotic transmission of malaria in the Atlantic Forest can vary depending on the abundance and acrodendrophily of the vector mosquito. The findings indicated that (i) an increase in the abundance of the vector in relation to the total number of blood-seeking mosquitoes leads to an asymptotic increase in the proportion of infected individuals at steady state and R0, (ii) the proportion of infected humans at steady state increases with increasing displacement of the vector mosquito between the forest strata, (iii) in most scenarios, *Plasmodium* transmission would not be sustained between mosquitoes and humans alone, implying that non-human primates play an important role in maintaining the transmission cycle. The proposed model contributes to a better understanding of the dynamics of malaria transmission in the Atlantic Forest.

**Hantavirus**

The Hantavirus Cardiopulmonary Syndrome (HCPS) is a zoonotic disease characterized by severe pulmonary involvement that leads to respiratory failure and cardiogenic shock (Pinto-Junior et al. 2014) and is associated with high lethality rates in Brazil (Brazilian Ministry of Health 2013), and its relationship with environmental variables was also studied by researchers of the University of São Paulo. The main hosts are rodents in the family Muridae/Cricetidae (Jonsson et al. 2010) and the virus is transmitted to humans via inhalation of aerosolized virus particles derived from the urine, saliva, and feces of infected rodents (Lee et al. 1981, Doyle et al. 1998).

In the American continent, and especially in Brazil, HCPS has increased considerably over the last decades, becoming a public health problem (Bi et al. 2008). Between 1993 and 2013, 1781 HCPS cases were confirmed in Brazil, of which 50.4% occurred in the Atlantic Forest biome, with an increase in the number of reported cases in the last years (SINAN 2018). The black-footed pygmy rice rat – *Oligoryzomys nigripes* (Oilers 1818) and the hairy-tailed bolso mouse – *Necromys lasiurus* (Lund 1840) are the main reservoirs in the Atlantic Forest for human HCPS cases of the *J. orthohantavirus* and the *A. orthohantavirus* viruses, respectively (Suzuki et al. 2004), both belonging to the *Andes orthohantavirus* genotype (King et al. 2018). Both rodent species have a high degree of adaptability to anthropogenic environments, such as pastures (Martin et al. 2012), crops (Goodin et al. 2006, Umetsu & Pardini 2007), and to native habitat edges (Santos-Filho et al. 2012), increasing their abundances in comparison to native forest habitats.

In this project, both rodent reservoirs and HCPS cases and their relationship with environmental determinants were analyzed. By using a large empirical dataset, the response of *O. nigripes* and *N. lasiurus* were modelled to changes in forest cover and forest edge density in the Brazilian Atlantic Forest. Reservoir rodent abundance was sampled at 104 sites of the Brazilian Atlantic Forest, with the highest abundance of *N. lasiurus* being observed in agricultural areas and in landscapes with a low amount of forest cover (11%). For *O. nigripes* most of the individuals were captured in fragmented areas and again in the landscapes with a low amount of forest cover (11%). For both species, higher the amount of forest cover, lower their abundance (Prist et al. 2021). The potential impacts of restoring native tree species abundance in the Atlantic Forest in accordance with the 2012 Native Vegetation Protection Law was also analyzed. The 2012 Law regulates minimum mandatory native vegetation protection on private lands in Brazil, which constitute 20% of the Atlantic Forest Biome. However, not all landowners respect this legislation. If the law were fully enforced, an additional 6,200,000 ha of vegetation would be restored. The results found that restoration would lead to a 43% decrease in the abundance of *N. lasiurus* across 44.3% of the Atlantic Forest. For *O. nigripes*, restoration would lead to a 90% decrease across approximately 44% of the entire biome. Considering the total areas in which decreases in both rodent abundances would occur, 2,760,645 million people would benefit from forest restoration, which represents 31.57% of the total population in the area (Prist et al. 2021). However, when cases of the disease are modeled, forest cover does not seem to matter as much, and the amount of sugarcane present in each municipality is the most important variable. This result hints that the landscape determines the abundance of rodents, but for a person to become infected, risk behaviors, which may be associated with the crops from this agricultural use, are essential and determining a person to be infected (Prist et al. 2016, 2017).

**Yellow Fever**

Not only does landscape structure determine the abundance of hosts and vectors, as seen in the examples above, it is also essential to determine the movement of infected or infectious hosts and vectors (Russell et al. 2006), being a key issue in determining the spatio-temporal dispersion of a disease. The Yellow fever virus (YFV), a mosquito borne Flavivirus, was studied within this concept. The YFV causes a hemorrhagic fever in about 200,000 people and 30,000 deaths annually (Garske et al. 2014). In South America, the virus circulates in a sylvatic transmission cycle among non-human primates and diurnal mosquitoes *Haemagogus* spp. and *Sabethes* spp., with spillover occurring when mosquitoes transmit the virus to humans (Thoisy et al. 2020). Non-human primates are the main hosts of YFV (Month 2001), with the lower monkey (Alouatta spp.) being the species most susceptible to the infection (de Azevedo Fernandes et al. 2021). Its death indicates the circulation of the virus in specific geographic locations and the immediate need for human population vaccination. YFV notification in non-human primates is called epizootic events (i.e., a disease event in an animal population, Almeida et al. 2014), and in Brazil it requires immediate report (Brazilian Ministry of Health, 2017).

The dispersion of YFV was analyzed by considering 243 georeferenced sites of PCR-positive dead monkeys (*A. caraya*) in São Paulo state. These nonhuman primates’ cases were analyzed using a
network approach to model the movement of the epizootic events in time and space. In this network analysis, epizootic events portrayed nodes and the goal was to establish potential links (dispersion) between each pair of nodes. Parameters that conditioned to the establishment of those links were the date of each epizootic event, the distance among the nodes, and the permeability of the landscapes' composition. Results obtained showed an incredible dispersion rate per day of YFV across landscapes. On average YFV could disperse 1.42 km per day, but it could be seen with larger movements, being up to 6.9 km/day. As expected, dispersions were five times longer in summer than in winter (1.2 vs. 0.22 km/day). Additionally, most dispersions (73%) occurred within a week after the arrival of YFV in the node, but in winter, they occurred within two or three weeks. Lastly, YFV disperses via roads nearby forest or along forest edges in interface with agriculture, water, and forestry. Important barriers for virus movement were the core of urban, agricultural, and forest regions (Prist et al. 2022).

Due to the high number of human cases and deaths and the high mortality in non-human primates (NHP) during the recent outbreaks of YF in Brazil, a theoretical agent-based model that simulates the dynamics of YFV transmission in a hypothetical forest fragment during epizootic waves, was developed (Medeiros-Sousa et al. 2022), as part of the recent BIOTA/FAPESP project “Study of potential vectors and ecological factors associated with the emergence and circulation of yellow fever in Brazil” (2018/25437-6), coordinated by Mauro Toledo Marrelli (FSP/USP).

This model contains individual agents representing mosquitoes, breeding sites, howler monkeys (Alouatta) and other vertebrate species living and interacting in an environment where the YFV arose. The simulations of the model aimed to investigate the isolated and interaction effects of important input parameters linked to mosquitoes (abundance, transmission competence, transovarial transmission), monkeys (transmission competence), environment (abundance of dead-end hosts) and hypothetical alternative hosts (useful life, viremic period, abundance and transmission competence) in the following outcomes: (i) maximum proportion of infected mosquitoes, (ii) proportion of dead monkeys and (iii) YFV persistence in the environment. Local and global sensitivity analyses were used to assess the influence of different sets of input parameter values on the outputs. The simulation results suggest that mosquito density is one of the main factors responsible for YFV amplification during epizootics and reinforce the hypothesis that the severity of an outbreak and persistence of the virus in the environment depend on complex interaction between different factors associated with vectors, hosts, and the environment (Medeiros-Sousa et al. 2022).

To understand the real impact of the yellow fever virus over the howler monkey population was pointed out as a priority subject for research, therefore in order to provide opportunity to collect real data about the density and occupation of the remaining population of Alouatta guariba in Atlantic rainforest conservation units of State of São Paulo after the 2016-2019 yellow fever outbreak, the BIOTA/FAPESP project “Evaluation of the impact of Yellow Fever Virus in non-human-primates in two conservation units and the potential of an enzootic cycle in State of São Paulo” (2019/20590-3) has been developed. In this study, the howler monkeys remain population has been accessed through observation in transects and bioacoustic approach, this information will be of fundamental importance for predicting further new yellow fever outbreaks, also it has been developed new techniques for the disease surveillance in wildlife and moreover, providing very important data about howler monkeys conservation status.

Mosquito Diversity in the City of São Paulo

The Metropolitan Region of São Paulo is one of the largest and most populous on the planet and the few remaining natural environments have been converted, much of them, into parks and conservation units that serve the population as leisure areas and have fundamental strategic functions for the preservation of important springs and remaining biodiversity. Among the various known environmental impacts of urbanization process and deforestation, there is one, little studied and extremely crucial considering the dimensions of its potential impacts on public health, which is the proliferation of mosquitoes and the pathogens transmitted by them in these areas bordering urbanization. These environments are potential refuges for wild mosquitoes and invasive mosquitoes, among which some mosquito vectors of epidemiological importance.

The study of the geographical distribution of mosquitoes in Brazil is still incipient. The fauna of our cities is little known, giving importance only to vector and provocative species of nuisance such as Aedes aegypti, Aedes albopictus and Culex quinquefasciatus (all exotic). Due to the scarcity of information about mosquito diversity in the city of São Paulo, in 2010, a first project funded by Biota program was initiated (“Mosquito biodiversity (Diptera: Culicidae) in the municipal parks of the city of São Paulo”, 2014/50444-5), with the objective of inventorying and developing a series of studies on the species of mosquitoes that inhabit the urban parks of the city of São Paulo, The drivers of mosquito richness and composition in urban parks in the city of São Paulo, Brazil were also investigated. Using the equilibrium theory of island biogeography, predictive models for species richness and composition and performed nestedness analysis were tested (Medeiros-Sousa et al. 2017). There is a limited discussion about the evolution of sinanthropy among the indigenous species of the country. Thus, this work allowed us to know a little about the geography of our urban mosquitoes and serve as incentives for the creation of lines of research in this direction.

After three years of investigation approximately 90 species of mosquitoes inhabiting these sites were identified (Medeiros-Sousa et al. 2017). Municipal parks, such as the Anhanguera park, had a wealth 57 of species richness (Medeiros-Sousa et al. 2015), while more central parks, such as the most famous and visited park of the city, Ibirapuera park, presented much lower wealth of richness with 16 species (Ceretti-Jr et al. 2015). Although the observed richness of mosquitoes can be considered relatively high at the regional level, about 70% of the individuals collected belong to five species (Medeiros-Sousa et al. 2017). The discovery of gaps in classical systematics was one perspective in a study of this nature. In the preliminary phase of this research, Anopheles fluminensis was found in one of the municipal parks (Carvalho et al. 2013). This finding surprised, because anophelines are generally sensitive to environmental changes and most of their species are susceptible to urban pollutants. The discovery of An. fluminensis in the City of São Paulo is important because it demonstrates some adaptation to the most urbanized and anthropogenic environments, which can influence changes in the epidemiological profile of malaria in the region, but the role of this species as a secondary vector remains unknown.
In a complement of this project, a second project was initiated, focusing efforts on areas susceptible to deforestation, in the green areas surrounding the city of São Paulo, the project “Mosquito Biodiversity (Diptera: Culicidae) in the Cantareira State Park and in the Environmental Protection Area Capivari – Monos, State of São Paulo” (2014/50444-5), developed a study of these species in the remaining areas of Atlantic Forest, in the metropolitan region of São Paulo. The Capivari-Monos Environmental Protection Area (EPA) and the Cantareira State Park (CSP) were the sites selected for the study due to the history of autochthon malaria in these areas, with cases of human malaria in the first and simian malaria in the second area. Another point that highlights the importance of the study is the hypothesis that these sites could function as a gateway so that arboviruses carried by mosquitoes that inhabit the wild environment can circulate among humans who inhabit periurban areas. This hypothesis was confirmed during the most recent and current epizootic outbreaks of YF in Brazil, which, as occurred in the two predecessors’ outbreaks, also expanded beyond the area of vaccine recommendation, with an alarming exacerbation of epizootics and human cases in the southeast of the country. From December 2016 to June 2018, 2,037 cases of YF were confirmed in Brazil, with 681 deaths. In the state of São Paulo, there were 864 epizootics in NHP from July 2016 to March 2018, and CSP was the entry of YF into the city (Fioravanti 2018). A publication with results of this project had already indicated that this would happen, when a total of 109 adult mosquito specimens and 30 immature forms belonging to 11 taxonomic categories in 4 genera (Aedes, Psorophora, Sabethes, and Haemagogus), including Hg. leucocelaenae, the main vector of yellow fever were collected in the CSP (Mucci et al. 2016, Ceretti-Júnior et al. 2020). These entomological findings had already indicated that the area is of strategic importance for yellow fever surveillance not only because of the significant numbers of humans and nonhuman primates circulating in CSP and its vicinity but also because it represents a potential route for the disease to be introduced to the SPMA.

In addition, during this project, the techniques used to collect mosquitoes achieved allowed a large number of niches in CSP to be explored, helping greatly to expand the knowledge of the culicid fauna in this region. Among the 11,038 specimens collected, at least 90 species represented by 16 genera were found. Before that, only the 22 Culicidae species had been reported in the same area and were included in the CSP Management Plan (Montes 2005).

Together, the results of these two projects on mosquito biodiversity suggest there is a species-area relationship, an increase in species similarity, as richness is lost and a nested species composition pattern. A few species, those most associated with the delivery of pathogens to humans, tend to benefit from such changes because they have the ability to develop in environments of the most diverse nature, including those artificially implanted by man (Medeiros-Sousa et al. 2017), suggesting a possible link between species loss and increased risk of pathogen transmission. The data highlight the need for studies that seek to understand how species loss may affect the risk of infectious diseases in urban areas. Human-altered environments tend to reduce the quantity and quality of habitats available for the development of immature forms and shelter for populations of many mosquito species. The reduction or lack of predators and natural competitors, in addition to the constant availability of sources of blood meals (humans and domesticated animals), has allowed these species to reach high abundance rates at certain times of the year.

In places where humans live with abundant mosquitoes and there is circulation of a pathogen there is greater exposure to mosquito bites, increasing the chance that one of them may be an infectious bite. Scenarios like this can be found in urban centers or even in newly degraded areas where there is the invasion and establishment of man. Currently, approximately 3,600 species of Culicidae (Harbach 2021) are described and validated, many of which form complexes of cryptic species. It is estimated that this amount represents only one quarter or a maximum of half of all mosquito species present in the wild. Many species will still be described, and research into the survey of mosquito biodiversity in the wild has been important to these investigations.

In the collection areas of both projects, the physical and chemical parameters of the water of the breeding sites and the ecological aspects related to the fauna of Culicidae were also analyzed. Regarding to the municipal parks, the results suggest ranges of conditions for the measured variables in which most mosquito species tend to be found more frequently, and pH and salinity are the variables most closely associated with variations in mosquito composition (Medeiros-Sousa et al. 2020). It is worth to note that *Ae. aegypti* and *Ae. albopictus* were present in both natural and artificial breeding sites and were observed under significantly varying conditions of pH, salinity, dissolved oxygen, and temperature. This information broadens our understanding of the ecology and interaction of the investigated species with abiotic factors in the aquatic environments, providing useful data for studies that seek to elucidate the underlying mechanisms of selection and colonization of breeding sites by these mosquitoes. This study also reinforces previous observations indicating that *Ae. albopictus* and *Ae. aegypti* can colonize diverse types of larval habitats with widely varying physical and chemical conditions.

Investigating physical and chemical parameters of the water of the breeding sites in the EPA and CSP also revealed a significant association between mosquito species occurrence and habitat pH (Multini et al. 2021). The type of aquatic habitat also had a significant influence on mosquito species distribution. pH values had a statistically significant influence on mosquito species occurrence and community composition, regardless of the type of aquatic habitat, suggesting that this parameter drives mosquito population dynamics and species distribution (Multini et al. 2021). Investigating the interactions between immature mosquitoes and the environment in which they develop is important to elucidate the factors driving their incidence and abundance and could be an important tool in planning and implementing immature mosquito control practices.

Mosquitoes were collected in immature and adult phases using various methods and then identified and separated for flavivirus analyses and investigation of blood meal sources of mosquitoes. The possible presence of arbovirus, mainly Flavivirus, in the collected mosquitoes was investigated and at the same time investigated the possible virological infections of birds that frequent or nest in the parks of São Paulo (Natal-Fernandes et al. 2016, Orico et al. 2016). The study failed to find any flaviviruses of medical importance in the municipal parks of São Paulo, however, nucleotide sequences of *Culex flavivirus* (CxFV) and *Aedes flavivirus* (AeFV) were detected in the mosquitoes (Natal-Fernandes et al. 2016). This was the first report of CxFV and AE芙V in the city of São Paulo and Latin America, respectively. Both viruses are insect-specific flaviviruses, a group known to replicate only
in mosquito cells and induce a cytopathic effect in some situations. It is strongly recommended that surveillance of flaviviruses in these locations be undertaken because of the risk of circulation of pathogenic viruses of this genus. In addition, the investigation of virological infections of birds were negative for the presence of viral ribonucleic acid (RNA) (Orico et al. 2016). Despite the negative results, Flavivirus surveillance must be performed regularly due to favorable ecological conditions for virus circulation and transmission among birds in these areas and their close proximity to humans.

Flavivirus investigation in the mosquitoes collected in EPA and CSP areas detected DENV serotype 2 (DENV-2) in Culex spp. and Culex vaxus, and ZIKV in An. cruzii, Limatus durhamii and Wy. confusa. Detection of flavivirus species of medical importance has never previously been reported in these species of wild-caught mosquitoes (Barrio-Nuevo et al. 2020). The findings suggest the existence of an enzootic cycle in the area. In-depth studies of DENV-2 and ZIKV, including investigation of mosquito infection, vector competence and infection in sylvatic hosts, are needed to shed light on the transmission dynamics of these important viruses and the potential risk of future outbreaks of DENV-2 and ZIKV infections in the region.

The investigation of blood meal sources of mosquitoes and their possible associations between mosquito species and their food preferences captured in municipal parks in the city of São Paulo, Brazil, revealed random patterns, indicating that the mosquitoes fed on the most abundant or convenient blood meal sources (Carvalho et al. 2014). Although feeding preferences were observed in two species (birds in the case of Cx. nigripalpus and dogs in the case of Cx. quinquefasciatus), our results highlight the opportunistic feeding habits of the female mosquitoes in this study. This deserves attention since changes in the transmission dynamics of mosquito-borne diseases can be triggered by the introduction of new hosts and pathogen reservoirs in the city of São Paulo.

Since little is known about how variations in mosquito diversity and feeding preferences for different hosts in different vegetation strata can influence the risk of pathogen transmission to humans, the vertical stratification of mosquitoes and its relationship with vertebrate hosts in environments with different degrees of conservation in two conservation units in the city of São Paulo were investigated (Evangelista et al. 2021). As results, species richness and composition tended to vary little between canopy and ground level in the same environment, but the abundance between canopy and ground level varied more depending on the species analysed, with the most abundant and frequent species exhibiting a predilection for the canopy. Even those mosquito species observed more frequently in the canopy did not show an association with hosts found in this stratum as most of the blood identified in these species was from humans, suggesting opportunist-feeding behavior, i.e., feeding on the most readily available host in the environment. Find out more about the contributions of BIOTA/FAPESP Program regarding terrestrial invertebrates in Noll et al. (in press).

**Spotted Fever**

This tick-borne disease is caused by the bacterium *Rickettsia rickettsii* which, in Brazil, is transmitted by two tick species, *Amblyomma sculputum* and *Amblyomma aureolatum* (Szabó et al. 2013). The species *A. aureolatum* is autochthonous from the Atlantic rainforest, and it is associated with spotted fever occurrence, when human beings dwell nearby remain forest areas (Pinter et al. 2004). In 2012 it was described, for the first time, that the higher incidence of the disease was correlated to loss of biodiversity within Atlantic rainforest remain patches, in fact, the etiologic agent was not detectable in areas with higher biodiversity (Ogrzewalska et al. 2012), thereafter it was demonstrated that the forest fragmentation pattern and, in special, the isolation of the forest patches with absence of functional ecological corridors is the key factor for occurrence of the spotted fever in humans (Scinachi et al. 2017). This happens because *A. aureolatum* uses wild carnivores as the natural host, but when the absence of forest connectivity prevents the occupation of wild carnivores, *A. aureolatum* ticks develop a surrogate cycle using domestic dog as the main host, as long as unrestricted maintained dogs have access to inner parts of the forest (Pinter et al. 2008), since domestic dogs shows a higher population turnover, when compared to wild carnivores, and, once infected by *R. rickettsii*, develop rickettsemia and become competent amplifiers of the bacterium, acting as a source of infection for susceptible ticks, what increasing in a large magnitude the number of infected ticks and therefore increasing the chance of a infected tick to feed upon a human being (Saraiva et al. 2014, Binder et al. 2021).

The recombination of functional and structural ecological corridors for wild carnivores may be a sustainable measure for prevent occurrence of spotted fever in humans in Atlantic rainforest areas.

**Bioagents in Birds**

Brazil is one of the countries with the highest biodiversity in birds in the world, many of these bird species have developed synanthropic behaviours, both in rural or urban areas, and therefore they are in close contact with human and domestic animals. Those bird species may carry and be a source of infection of several sort of pathogens, as well as to be infected by agents from livestock animals. BIOTA/FAPESP project 11/50919-5 – “Investigation of viral agents in biota of wild birds in the State of Sao Paulo”, coordinated by Clarice Weis Arns (UNICAMP), has been conducted with the main goal of evaluating this scenario. In fact, some species have been found infected as a product of this project. Data about the phylogenetic analysis and dynamic of avian coronavirus (AvCoV) was published by Durães-Carvalho et al. (2015), among 42 accessed bird species, 22 different bird species distributed in 10 orders and 13 bird families had AccoV genetic material detected through tracheal and cloacal swabs, in special the families Anatidae, Ardeidae, Cathartidae, Columbidae, Falconidae, Icteridae, Passeridae, Phalacrocoracidae, Picidae, Psittacidae, Ramphastidae, Strigidae, and Tyrannidae. What shows a high prevalence of AvCoV circulation in birds, other than chickens.

Martini et al. (2018) tested an isolated AvCoV from a pingeon sample to verify the competence of infect chicken, it was demonstrated then, that this coronavirus strain can infect and produce disease in chicken, this study brought attention of the possible role played by non-galliformes in ecology of AvCoV.

These studies show a high concern in the circulation of AvCoV in wild and livestock birds, this poses an important threat for wildlife conservation, and must be of special attention for potential spill over events that may onset outbreaks in the human population.
Birds also carry ectoparasites, the project BIOTA/FAPESP 11/50145-0 – “Diversity and taxonomy of feather mites (Acachnida: Acari: Astigmata) in birds in Brazil”, coordinated by Fábio Rau Akashi Hernandez (IB/UNESP-Rio Claro) had the access and characterization of feather mites in birds as the main goal. Several mite species use passeriform birds as hosts, and despite the immense biodiversity both of mites and birds, these organisms still have much yet to be studied. This project also aimed to create a scientific collection of reference for mites, to both morphological and genetic purposes. As expected, this project turned out to produce more than 30 scientific articles about the characterization, description and redescription of feather mite species. As a few examples there are: the discovery of new mite species published by Hernandes (2017) described two new species of the genus Trouessartia parasitizing Philydor atricapillus (wied) and Tyranus melancholicus (Tyrianus).

A new species of the mite genus Proctophyllodes was collected from tanger birds (Hernandes et al. 2016). In the study, besides new species of mites, a new genus was also discovered, as described by Hernandes & Mironov (2015), mites collected on hoatzin Opisthocomus hoazin were described into the new proposed genus Gymnolichus (Acari: Psoroptoididae).

Those projects and publications yield the conclusion that to research and to describe parasite biodiversity is a subject that must receive full effort from the research community, and much remains to be learned and discovered.

**Biodiversity of Venomous Animals**

Besides the diseases caused by infectious agents, a public health main issue is the accident with venomous animals, moreover, to develop research on the biochemical structure of the venom may be useful in the production of the medical components for therapeutic use.

The project BIOTA/FAPESP 16/50127-5 – “Dimensions US-BIOTA São Paulo: scales of biodiversity: integrated studies of snake venom evolution and function across multiple levels of diversity”, coordinated by Inácio de Loiola Meirelles Junqueira de Azevedo (Instituto Butantan-SP) presented as the main goal to describe the genotypic diversity of venoms from different snakes’ species and the correlation with genetic evolution. To reach this objective, venom samples from more than 100 species of snakes collected in North and South America were analyzed to the molecular level. From this project, more than 20 scientific publications were released, in special.

Sousa et al. (2021) showed a high variability in the composition of the venom from different species of Bothrops (pit viper) collected in four different locations in the Amazon biome, the authors support the hypothesis that the differential distribution of protein isoforms results in functional distinctiveness and the ability of snakes with different venoms to have variable toxic effects on different prey.

Gibbs et al. (2018) demonstrated an important genetic divergence among populations of the pitviper Bothrops atrox in different locations in the Amazon Forest, suggesting a role for the Amazon River as a driver of *in situ* divergence both by partially impeding gene flow and through parapatric differentiation along an ecological gradient.

The project also allowed collaboration with researchers from different groups, for example, the analysis of the collected ectoparasites was made alongside mites and ticks specialists, as described by Mendoza-Roldan et al. (2020) that published a list of parasites found on snakes in Brazil. Species Amblyomma ticks and Trombiculiforme mites were found on snakes. This article shows the importance of collaboration among researchers and the potential expansion of a project beyond the primary objective.

**Perspectives for the Knowledge Area/Topic in the Next 10 Years of the BIOTA Program**

The increment of the knowledge about biodiversity is an important tool for better understanding the potential of emergence and propagation of etiological agents that may cause diseases and other health problems.

For the next 10 years, the modification of the landscape and climate variables must be a main point of focus for researchers.

Both suppression and recuperation of the forests may cause an unbalanced situation among vertebrate and invertebrate animals and potential pathogenic agents that may produce diseases and other health problems to the human being.

To study and determine hotspots locally where, more likely, emergence or reemergence of diseases events may happen must be a priority.

The biodiversity studies aiming to describe the abundance, richness and occurrence of vectors and etiological agents have been demonstrated as of maximum importance. Mosquitos, kissing bugs, mites, ticks, sand flies, and other groups are under direct pressure of environmental and climate changes. To understand how these changes will influence human public health must be encouraged.

The projects on mosquito biodiversity in São Paulo city effectively contributed to the conservation and sustainability of São Paulo State’s biodiversity. The proposals of these projects were outlined in the search for information on the resilience of mosquito species in the stressed environments of the cities and protected areas. The species, some of them little known or rare, which have resisted environmental changes surviving islanders in the parks can be used as indicators of stressed environments. In addition to this contribution, the simple knowledge of diversity within these islands will show the degree of human interference in these restricted environments. The survey of the biodiversity of this group of insects in the urban environment and environmentally protected areas of a metropolis such as São Paulo opens perspective to know which are the autochthon species that resisted the process of urbanization and persisted in the highly modified human environment and also the existence and behavior of mosquito invasive species.

Vertebrates, mainly rodents and birds are potential reservoirs of infinity of viruses that can be pathogenic to humans. The knowledge generated by the projects on mosquito biodiversity, combined with studies of vertebrates included in other BIOTA FAPESP projects will help to assess the potential risk of emergence of arboviruses in the urban environment.

Determination of areas of high priority for biodiversity conservation and areas where functional and structural ecological corridors should be implemented or expanded must be considered an important goal for research, in order to increase biodiversity, which has been shown as an approach for preventing potential further public health issues.
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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

This study did not involve human beings and/or clinical trials that should be approved by one Institutional Committee.

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