ABSTRACT

The Mosquito Quest

The fight against vector-borne diseases represents one of the biggest and most significant challenges to the current and future human wellbeing. In our project, we build a device for near real-time monitoring of vector borne diseases, to implement a new, large-scale surveillance system that could ultimately help to reduce the spread of arboviral illnesses throughout the Earth.

Our Global Arbovirus Mapping System, GAMs, relies on two main components: firstly, Global Arbovirus Mapping units, GAMu, a network of ground-based detection stations, located in mosquito-adequate hotspots. Secondly, the Global Arbovirus Mapping Platform, an unprecedented system of mapping and profiling of viral species and variants distribution through near real-time automated data transmission. The device used as a surveillance station is the core unit for data collection and processing: it obtains biological material directly from the mosquito bite; then, it analyses the viral genome with portable sequencing technologies, and ultimately it both stores the data locally and transmits the relevant information to the local, regional and national health centers. This way, we adopt an engineering solution to build a device that fully exploits the power of the new, portable genome sequencing technologies, combined with an IoT approach.

The pilot phase will see first of all the construction of the prototype. The testing of the GAMu will then be in the Barrio of Manguinhos in the city of Rio de Janeiro. It will be functional to the amelioration of the GAMu and the appropriate targeted measures, as well as the coordination between the device and the local health facilities. Also, the pilot phase will allow us to precisely estimate the cost-benefit ratio before the scaling up. The final idea is to scale the GAMu globally to all regions at risk or with endemic presence of arboviruses, and to create a Global Arbovirus Mapping System, allowing access to health facilities globally to near real-time data, helping the WHO, national and regional health systems anticipating and preventing the development of epidemics.

The beneficiaries would be first of all the populations residing in areas affected by various types of arboviruses, as well as those at risk of infection, but also national governments, since the device is intended to be a tool capable of reducing the costs associated with the prevention system and increase the efficiency and effectiveness of each individual intervention, and the International Community, thanks to this unique data-sharing system.

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THE GRADUATE INSTITUTE - IHEID

The Mosquito Quest

Global Arbovirus Mapping system:
looking for an innovative monitoring and surveillance system of arboviruses in the global context

GENEVA CHALLENGE - 2019 EDITION

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The Mosquito Quest

The fight against vector-borne diseases represents one of the biggest and most significant challenges to the current and future human wellbeing. In our project, we build a device for near real-time monitoring of vector borne diseases, to implement a new, large-scale surveillance system that could ultimately help to reduce the spread of arboviral illnesses throughout the Earth.

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1.Introduction

Malaria, West-Nile virus, Yellow fever, Japanese encephalitis, as well as Dengue, Leishmaniasis, Human American and African trypanosomiasis are nowadays widespread diseases, transmitted by various vectors, of which the mosquito is the primary one[1]. Overall, these diseases have severe impacts on many tropical and subtropical countries, where they are responsible for around 10% of human deaths and impose a burden of more than 50 million disability-adjusted life years (DALYs) annually[2]. Also, vector-borne diseases are becoming a serious health-concern for more developed countries, such as Europe, because of the expansion of the geographic distribution of vectors in response to climate change[3], or accidental introductions of vectors and pathogens through international migration and commercial exchanges[4]. Vector control is therefore extremely challenging, and the solutions implemented until now have not proven effective enough to stop the vector threat.

1.1. Arboviral diseases

Vector-borne diseases are human and animal illnesses caused by parasites, viruses and bacteria that are transmitted by numerous insects; together, they account for more than 17% of all infectious diseases, causing more than 700.000 deaths annually[5]. Among vector-borne illnesses, **arboviral diseases** play a major role in threatening global health. Their name reflects their origin, as they are diseases caused by arthropod-borne viruses. Today, the most common vectors carrying arboviruses are **mosquitoes**, counting approximately 300 species that can transmit these diseases[6]. More specifically, *Aedes* and *Culex* species of mosquitoes are the most frequently associated with arboviral diseases, transmitting 115 and 105 types of arbovirus respectively[7]. Indeed, *Aedes* spp. distributions are now the widest ever recorded, with more than 3 billion people living in *Aedes*-infested regions[8]. Knowledge of the contemporary distribution of the arboviral vectors remains incomplete and is further complicated by global trade and travel, unplanned urbanization and climate change, impacting on pathogen transmission and causing the emergence of diseases in new countries.

For these reasons, **mapping** the global distribution of these vectors and the geographical determinants of their ranges is essential for public health planning. Indeed, vector elimination cannot be effectively and efficiently carried out without a reliable and timely **surveillance system**. As shown more in depth in **Paragraph 2.4**, most current surveillance methods lack a timely identification of viral genome, require continuous human interventions and are not integrated in a real-time dynamic map monitoring of viral spreading.

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[1] Hotez PJ, Molyneux DH, fenwick A, Kumaresan J, Ehrlich Sachs S, et al. (2007) Control of neglected tropical diseases. N Engl J Med 357: 1018–1027.
[2] Hotez PJ, Molyneux DH, Fenwick A, Ottesen E, Ehrlich Sachs S, et al. (2006) Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med 3: e102.
[3] Martens WJ, Niessen LW, Rotmans J, Jetten TH, McMichael AJ (1995) Potential impact of global climate change on malaria risk. Env Health Perspect 103: 458–464.
[4] Enserink M (2008), A mosquito goes global. Science 320: 864–866.
[5] WHO Fact Sheet, Vector-Borne Diseases (https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases)
[6] Liang, G., Gao, X., Gould, E.A., Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control, Emerging Microbes and Infections (2015) 4, e18; doi:10.1038/emi.2015.18; published online 25 March 2015.
[7] *Ibidem*.
[8] Wilder-Smith, A., Gubler, D.J., Weaver, S.C., Monath, T.P., Heymann, D.L., Scott, T.W., Epidemic arboviral diseases: priorities for research and public health, *Lancet Infect Dis* 2017; 17: e101–06, published Online December 20, 2016, http://dx.doi.org/10.1016/ S1473-3099(16)30518-7.

In line with the 8th priority activity for 2017-2022 according to the WHO Global Vector Control Response[9], this project focuses exactly on the need for **strengthened national vector surveillance systems**, that need to work synergistically with health information systems, to guide more effective vector control led both by viral spread and disease epidemiology.

1.2. Project scope

The aim of this ambitious project is to offer a scalable solution to increase the efficiency of existing vector surveillance systems through the **Global Arbovirus Mapping System** (GAMs), a near real-time, automated monitoring the dynamics of viral species and variants.

1.2.1. GAMu: the technology

The **Global Arbovirus Mapping unit** (GAMu), our unit device, will allow constant and unsupervised sampling of virome from mosquito saliva in rural and urban areas. It will interact with the vector on-site, collect the **sample, process the genome processing and transmit the data.** We plan to build a device that combines electromechanical components with new technologies for genomic analysis, merging them in an extremely creative (but still scientifically feasible) product.

1.2.2. GAMs: project implementation

The ultimate goal of collecting and integrating such data would be producing and giving to the disposal of sovranational health-promoting institutions, through the Global Arboviruses Mapping Platform, continuously updated dynamic maps of vector-borne viruses diffusion and movement through space, which would not only be informative about viruses fitness and virulence, but also useful for coordinating middle- or short-term preventive interventions towards potential epidemic outbreaks.

The final goal is to anticipate preventive interventions, making them more effective and timelier, thus reducing the risk of epidemic outbreaks and mosquito-borne diseases cases, therefore improving the economic and social development of the countries affected by vector transmitted diseases.

GAMs might prove an essential technology to achieve the target 3.3 of the Sustainable Development Goals Framework, which aims at ending epidemics of communicable diseases by 2030.

[9] World Health Organization. (2017). *Vector control* (No. SEA/RC70/10). World Health Organization. Regional Office for South-East Asia.

2.The Problem: Arboviruses as Global Health Challenge

2.1. Scientific base of arboviruses transmission

Arthropod-**bo**rne viruses (arboviruses) are transmitted biologically among vertebrate hosts by blood feeding arthropod vectors such as mosquitoes, other biting flies and ticks[10]. The wide and epiphenomenal definition of this group allows the inclusion of many viruses with conspicuous differences from each other in terms of biology and human risk profile. Indeed, the arboviruses include a wide variety of **RNA virus taxa**, counting several genera in the family *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Reoviridae*, *Rhabdoviridae* and *Orthomyxoviridae*[11]. These groups of RNA pathogens represent a significant infective threat to the human immune system, since they rely on several RNA replication strategies, including the generation of new genomic variants with their replication[12].

Being, by definition, biologically transmitted, arboviruses must replicate in the arthropod vector prior to transmission, as opposed to being mechanically transmitted, without replication in the vector, through contaminated mouthparts[13]. Arboviruses usually circulate among wild animals, therefore can cause human illnesses after spillover transmission to humans and/or domestic animals, which are often *accidental hosts.* Also, after the infection sometimes viruses cannot be transmitted from men or domestic animals, that in this case are *dead-end* hosts. The replication cycles of common arboviruses are shown in **Figure 2.1.a**.

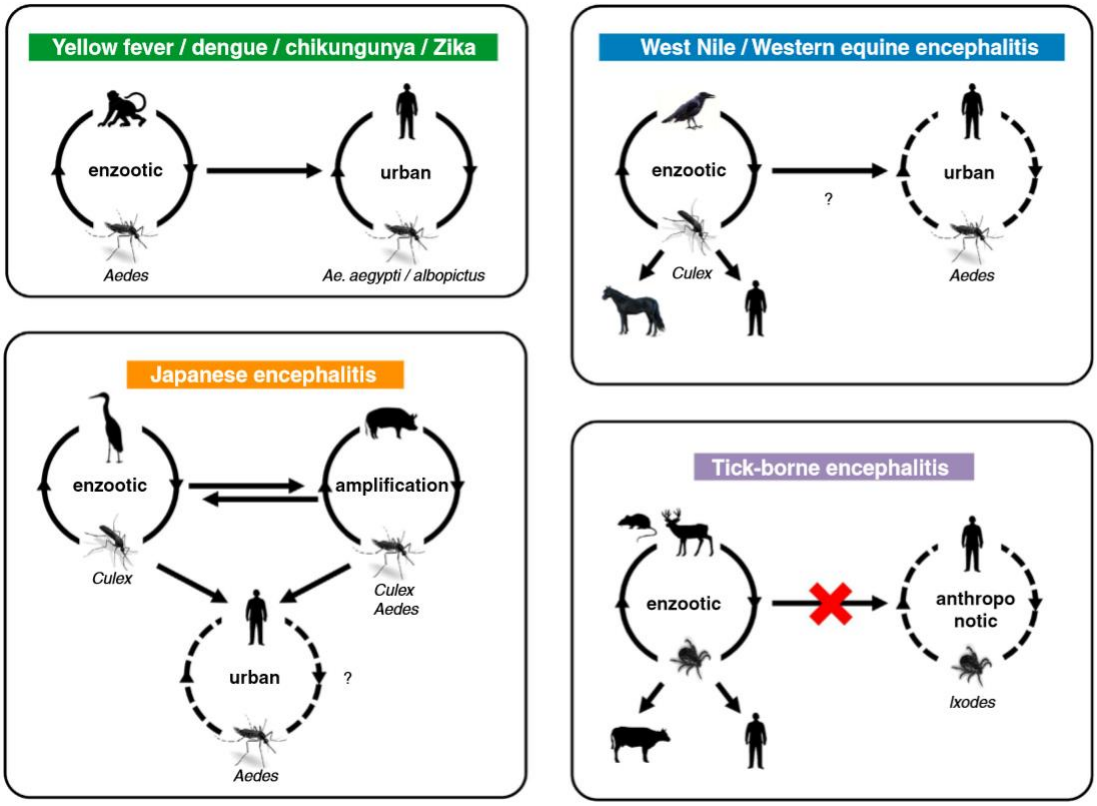

Figure 2.1.a: Examples of different arboviruses transmission cycles. From **Martina et al, 2015**[14].

[10] Weaver, S.C., Reisen, W.K., Present and Future Arboviral Threats, *Antiviral Res.* 2010 February ; 85(2): 328. doi:10.1016/j.antiviral.2009.10.008.
[11] *Ibidem.*
[12] *Ibidem.*
[13] Weaver, SC. Vector Biology in Viral Pathogenesis. In: Nathanson, N., editor. Viral Pathogenesis. Lippincott-Raven; New York: 1997. p.329-352.
[14] Martina, B. E., Barzon, L., Pijlman, G. P., de la Fuente, J., Rizzoli, A., Wammes, L. J., … Papa, A. (2017). Human to human transmission of arthropod-borne pathogens. Current Opinion in Virology, 22, 13–21. doi:10.1016/j.coviro.2016.11.005

The ability of these viruses to cause human disease depends on factors ranging from epidemiology to viral genetics[15], as shown in **Figure 2.1.b**. All arboviruses are transmitted in zoonotic cycles involving **non-human primates** and **arboreal mosquitoes**, and have entered human-to-human cycles involving mainly urban *A. aegypti* and, in some cases, *A. albopictus* transmission[16]. *Aedes spp* distributions are now the widest ever recorded and extend in all continents, including North America and Europe, with more than 3 billion people living in aedes-infested regions[17]. ***A. aegypti***, the principal vector, is mainly spread in tropical and subtropical areas.

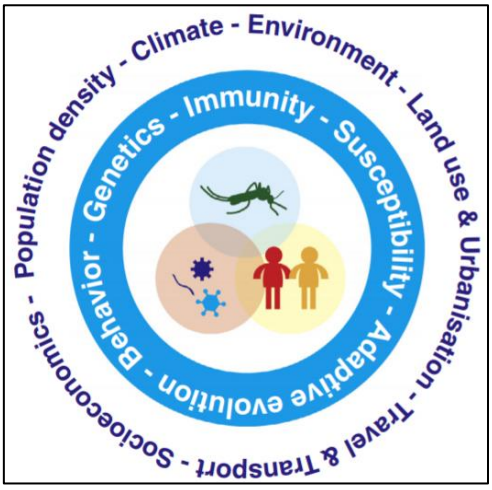

Figure 2.1.b: Different factors influencing human-to-human viral transmission and infection in arbovirus diseases.
From **Martina et al, 2015**[18].

It feeds preferentially on human beings and is one of the most efficient vectors for several reasons, including its **high susceptibility** to infection by arboviruses, its activity during the daytime, its spreading **in peridomestic environments** close and the **high frequency of its biting**[19]. Furthermore, overcrowding facilitates transmission via urban *Aedes* mosquitoes, further compounded by man-made larval habitats, suggesting that one of the principal drivers of *A. aegypti*-borne diseases is the unprecedented urbanisation in tropical low-income countries in the past 50 years[20]. Furthermore, increasing international travel and globalisation accelerate the introduction of arboviruses into new areas and their geographic expansion.

[15] Weaver, SC. Vector Biology in Viral Pathogenesis. In: Nathanson, N., editor. Viral Pathogenesis. Lippincott-Raven; New York: 1997. p.329-352.
[16] Wilder-Smith, A., Gubler, D.J., Weaver, S.C., Monath, T.P., Heymann, D.L., Scott, T.W., Epidemic arboviral diseases: priorities for research and public health, December 20, 2016 http://dx.doi.org/10.1016/ S1473-3099(16)30518-7.
[17] *Ibidem.*
[18] Martina, B. E., Barzon, L., Pijlman, G. P., de la Fuente, J., Rizzoli, A., Wammes, L. J., … Papa, A. (2017). Human to human transmission of arthropod-borne pathogens. Current Opinion in Virology, 22, 13–21. doi:10.1016/j.coviro.2016.11.005
[19] *Ibidem.*
[20] *Ibidem.*

2.2. Impact on the global population health

According to the WHO, every year more than one billion people are infected, and more than one million people die from vector-borne diseases including Malaria, Dengue, Schistosomiasis, Leishmaniasis, Chagas disease, Yellow Fever, lymphatic filariasis and onchocerciasis[21] (**Figure 2.2**). The major vector-borne diseases together account for around **17% of all infectious diseases**, with the burden of these illnesses carried mostly in tropical and subtropical areas, disproportionately affecting the poorest populations[22]. The affected communities are mostly urban, peri-urban and rural, but VBD thrive predominantly among communities with **poor living conditions**, in particular because of the lack of access to adequate housing, safe drinking water and sanitation; consequently, malnourished people and those with weakened immunity are the part of the population especially vulnerable to these viruses[23]. Over one sixth of the illness and disability suffered worldwide is due to vector-borne diseases, with more than half the world's population currently estimated to be at risk of these diseases[24].

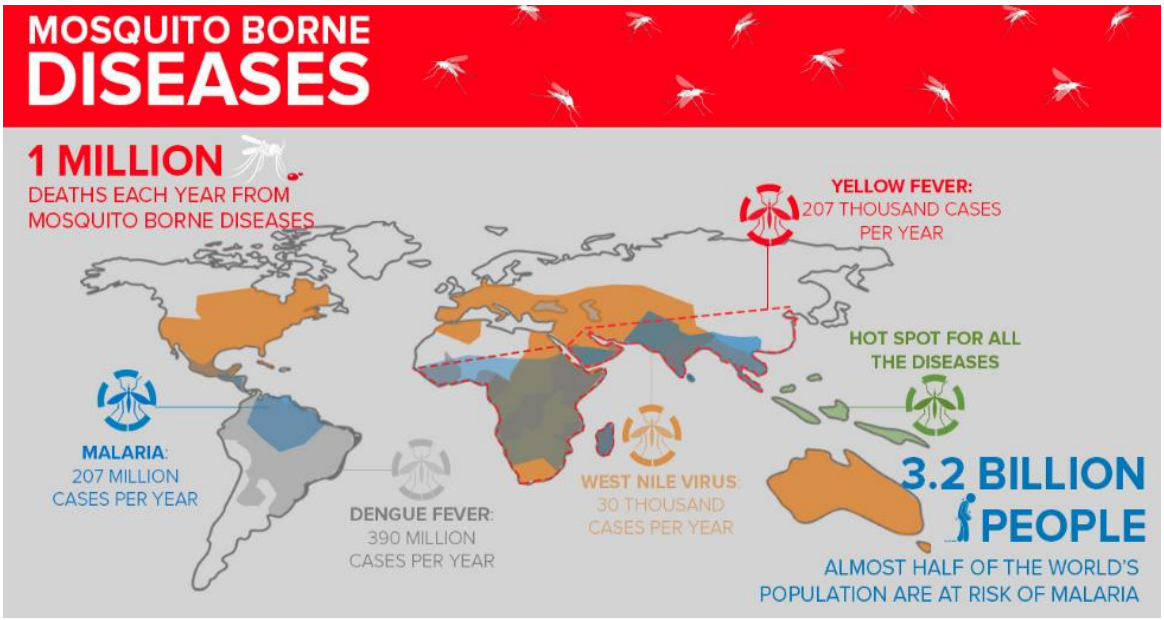

Figure 2.2: Estimates of people affected by VBD in 2015[25].

The **Sustainable Development Goals (SDGs) Framework** addresses specifically the burden of communicable diseases in Target 3.3, aiming at ending by 2030 the epidemics of malaria, neglected tropical diseases and other communicable diseases, including VBD. Moreover, reducing VBD goes beyond ensuring good health and well-being, since it could be an important driver also for interventions for clean water and sanitation (Goal 6), sustainable cities and communities (Goal 11), poverty reduction (Goal 1), and climate action (Goal 13)[26].

[21] WHO, A global Brief on vector-borne diseases, document number: WHO/DCO/WHD/2014.1, WHO, 2014.
[22] WHO Fact Sheet, Vector-Borne Diseases (https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases)
[23] WHO, A global Brief on vector-borne diseases, document number: WHO/DCO/WHD/2014.1, WHO, 2014.
[24] WHO, The world health report 2004, Changing history, Geneva: World Health Organization, 2004.
[25] https://www.rentokil.com/blog/mosquito-borne-diseases/
[26] WHO, Global vector control response 2017–2030. Geneva: World Health Organization; 2017.

2.3. Economic burden of mosquito-borne diseases

The economic burden of vector-borne diseases to society is substantial. Direct costs of mosquito borne diseases are supported by **governments** and **international organizations**, particularly in endemic countries, and by **households**. The firsts are responsible for vector control activities and case management, whereas the seconds face expenditures related to personal protective measures and/or treatment, as well as foregone income due to reduced productivity or time off work due to illness or caregiving to sick household members[27]. Government direct costs consist in **financing preventive interventions, surveillance methods, and running and maintenance of health structures and workforce**. As an example, just for Dengue virus, a total annual global aggregate cost of US$ 8.9 billion was sustained for treating cases in 2013[28].

From a macroeconomic perspective, vector-borne diseases have been associated with **lower economic development**. The economic burden of VBD is not limited only at direct costs of health treatments, health structures, preventive interventions and surveillance methods. The impact of VBD is evident also on the poverty levels of a country: illness and disability prevent people from working and supporting themselves and their family, causing further hardship and impeding economic development. For example, the WHO estimates that contracting Dengue imposes a substantial economic burden on families and governments, both in terms of medical costs and in working days lost due to illness[29]. According to the study, an average dengue episode represents 14,8 lost days for ambulatory patients at an average cost of US$ 514 and 18,9 days for non-fatal hospitalized patients at an average cost of US$ 1.491[30].

[27] WHO, Global vector control response 2017–2030. Geneva: World Health Organization; 2017.
[28] Shepard, D. S., Undurraga, E. A., Halasa, Y. A., & Stanaway, J. D. (2016). The global economic burden of dengue: a systematic analysis. *The Lancet infectious diseases*, *16*(8), 935-941.
[29] WHO, Global strategy for dengue prevention and control, 2012- 2020, Geneva: World Health Organization; 2012
[30] *Ibidem.*

2.4. Current surveillance methods for mosquito-borne diseases

Surveillance is critical for prevention, control and management of mosquito-borne diseases. In fact, the detection of elevated or emergent virus activity serves as an efficient warning system to implement appropriate actions to reduce outbreaks[31]. However, designing an appropriate arbovirus surveillance system is challenging. Arboviruses have complex transmission cycles with dual-host tropism, replicating in vertebrate hosts, such as birds or mammals, and arthropod hematophagous vectors[32]. This complexity needs to be accounted for, and an ideal surveillance system should rely on **different sources of information**, and including meteorological data, evidence of virus infection in vertebrate hosts, entomological surveys, virus detection in vectors, and reports of human or animal disease. The level of **scaling** of surveillance systems can vary regionally; furthermore, an efficient monitoring can be particularly challenging to achieve in **remote locations**, or in areas with limited resources and infrastructure.

Different surveillance systems are used in different contexts, such as human or animal case surveillance, relying on hospital, laboratories and health practitioners notifying public health authorities of confirmed or suspected cases of arbovirus infection that occur in the population[33] or sentinel animals, providing evidence of virus activity and increased risk to target animal or human population[34], as shown in **Annex I**.

2.5. Needs and opportunity assessment

As shown in **Annex I**, surveillance methods adopted in several continents rely on the **reports of suspected cases** from healthcare providers, who submit blood samples from infected patients[35]. However, this kind of surveillance system presents the major limitation of being subject to the efficiency of the local health facilities, that may lack of diagnostic capability, especially in underdeveloped regions. Also, this solution only allows to collect information mainly from infected humans in urban areas, therefore reducing the scope of disease monitoring to the most crowded areas.

Alternatively, other solutions focus directly on **vector-based surveillance**: mosquitoes are collected, identified, pooled (usually by species) and sent to the laboratory, where they are manually tested for virus infection status[36]. There are different strategies for mosquito collection, depending on the level of mosquito infections: regions with low-level mosquito infections focus their surveillance efforts at "**hotspots**" with high likelihood of arbovirus presence; the number of sampling sites is expanded only if the viral infections increase[37]. There are numerous kinds of traps that catch mosquitoes and preserve them; manual labor is required for the traps to be emptied and the organic material to be analysed in laboratories. Indeed, this process can be highly **labor-intensive** since the traps must be checked periodically; once emptied, the insects are pooled by species or by other taxonomic group, and then the genomes from viruses and mosquitoes are analysed.

[31] Ramírez et al., Parasites & Vectors (2018), Searching for the proverbial needle in a haystack: advances in mosquito-borne arbovirus surveillance, 11:320 https://doi.org/10.1186/s13071-018-2901-x.
[32] Weaver, S.C., Reisen, W.K., Present and Future Arboviral Threats, *Antiviral Res*. 2010 February ; 85(2): 328. doi:10.1016/j.antiviral.2009.10.008.
[33] Ramírez et al. Parasites & Vectors (2018), Searching for the proverbial needle in the haysack: advances in mosquito-borne arbovirus surveillance, 11:320 https://doi.org/10.1186/s13071-018-2901-x
[34] Halliday JEB, Meredith AL, Knobel DL, Shaw DJ, Bronsvoort BMC, Cleaveland S. A framework for evaluating animals as sentinels for infectious disease surveillance. J R Soc Interface. 2007;4:973–84.
[35] Scarpino, S. V., Meyers, L. A., & Johansson, M. A. (2017). Design strategies for efficient arbovirus surveillance. *Emerging infectious diseases*, *23*(4), 642.
[36] Ramírez et al., Parasites & Vectors (2018), Searching for the proverbial needle in a haystack: advances in mosquito-borne arbovirus surveillance, 11:320 https://doi.org/10.1186/s13071-018-2901-x.
[37]Gu W, Unnasch TR, Katholi CR, Lampman R, Novak RJ. Fundamental issues in mosquito surveillance for arboviral transmission. Trans R Soc Trop Med Hyg. 2008;102:817–22.

Also, for time reasons very often the pools of mosquitoes are homogenized and analyzed together, only allowing to detect broadly the viruses carried by the pool of vectors, without keeping track of the number of infected mosquitoes nor how often a virus is encountered. Moreover, the mosquito's infection rate can be as low as 1/1000 even in endemic areas, therefore most of dead mosquitoes will not carry useful information about the virus.

Ideally, a large-scale system to **track the viral movements within vectors** would help mirroring the dynamics of human infection. Climate change and other factors are posing the significant challenge of expanding the areas of diffusion of vector-borne diseases, therefore a **mapping of viral variant diffusion** in urban, rural and emerging hotspots (such as the Mediterranean coast), could help monitoring the viral threats and *anticipate* their diffusion and epidemic outbreaks, while also generating useful data for **mathematical modelling.**

To our knowledge, none of the existing methods allow to integrate the process of sample collection directly from the vector, genome analysis and data transmission**; for this reason, this project focuses on developing an automated, mosquito-based arbovirus surveillance system, based on near real-time monitoring of vector populations and virus infection across the mosquito population.**

3.The Solution: GAMs – Global Arbovirus Mapping system

3.1. GAMu: device overview

We propose to create a network of fixed units, to be installed in areas of interest, that will allow **near real-time monitoring** of viral (and, potentially, mosquitoes) species in an automated way, integrating the processes of sample collection, genomic analysis and data transmission. In this Section, we explain how the device is structured and how its different components are made more in detail. The main revolutionary impact of our GAMu device is that it allows to obtain genomic information in a near real-time, automated way. At this stage, we do not intend to produce a detailed prototype, but we underly the fundamental metrics to prove that the device is feasible. Also, we pinpoint the critical issues to be considered for the device implementation, that will be tackled in the **prototyping phase** of the project development **Timeline**.

Overall, our device will be composed of three main parts:

1. A **sample collecting layer**, that will be made up of a chemoattractant-enriched membrane coupled with a UV light to attract mosquitoes, linked to a small fluidic system, conveying the mosquito saliva to the next layer via a syringe-pump and alimented by a refilling reservoir;
2. A **genome sequencing layer**, where the saliva sample is prepared, and the genomic sequences of the virus and, potentially, of the vector, are processed and analyzed through a small, portable system;
3. A **data storage and transmission layer**, to stock the full data in a memory and send relevant data to the platform via satellite.

A scheme of the device functioning is provided in **Figure 3.1**.

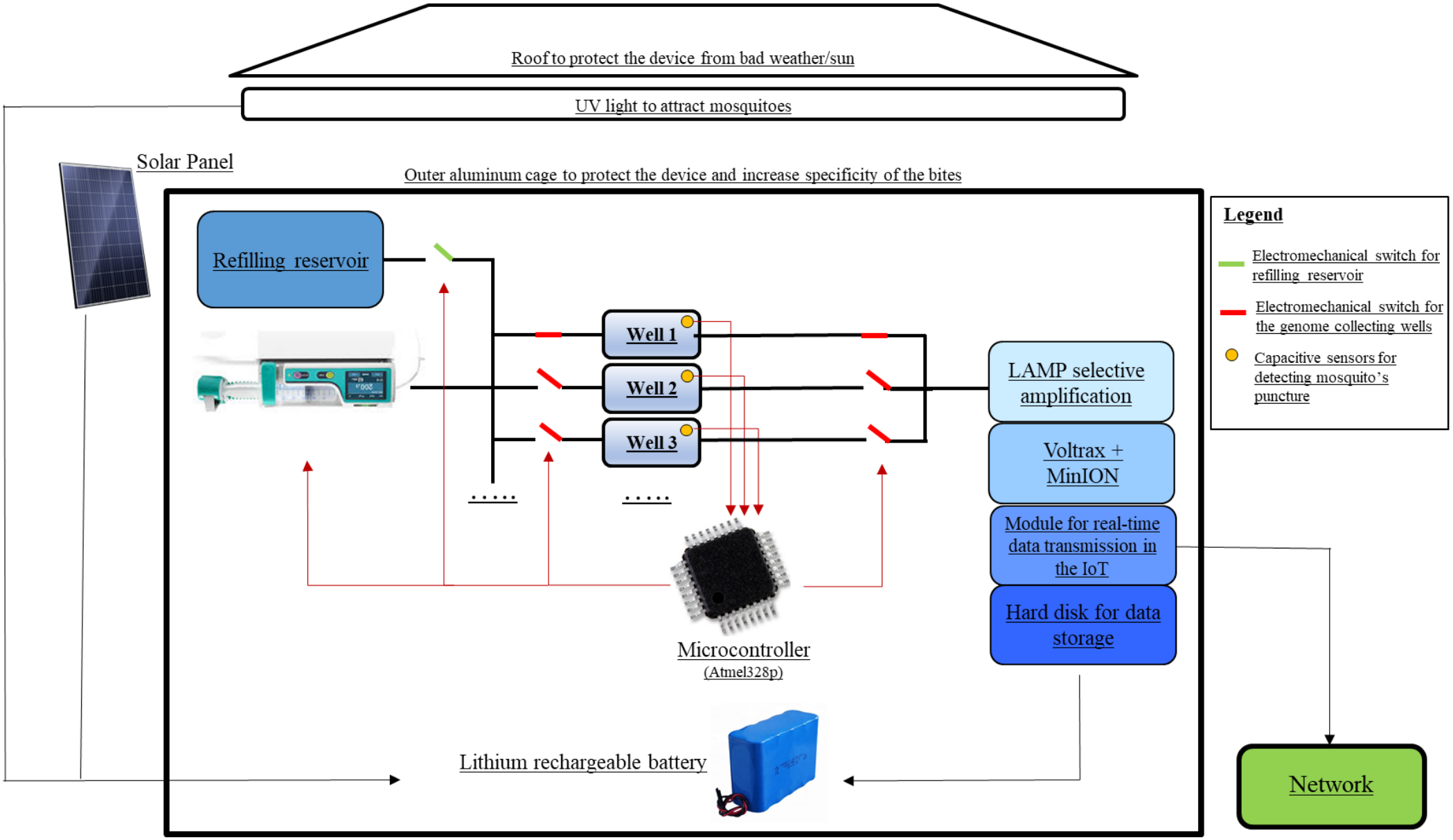

Figure 3.1: The Global Arbovirus Mapping unit. Scheme of the device functioning. A sample of saliva is collected from the mosquito bite and sent to the processing and analyzing part of the device. Finally, the information about the full viral genome is stored and a flag of the identified virus is immediately sent to the network via satellite.

3.2 First layer: sample collection

To attract mosquitoes and get the saliva sample, we plan to build a refillable circuit of fluid-filled compartments covered by a chemoattractant enriched membrane, as shown in **Figure 3.2**.

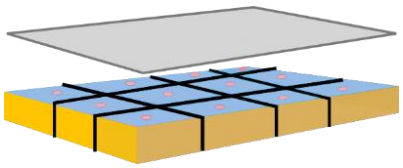

Figure 3.2: membrane-covered wells.

3.2.1 Attracting the mosquito

Among the sources causing the attraction of mosquitoes for human skin, there is a huge variety of cues, including odor plums combined with thermal targets and texture information[38], as well as UV light, which has extensively been used for low-cost, commercially available mosquito traps. Some research projects have been performed to identify the combination maximizing the landing probability of a mosquito; currently, a **parafilm** membrane[39] has proven a feasible solution in terms of favoring mosquito attraction and biting, therefore we plan to use it for our device. Also, we intend to put a small roof on our GAMu, both to protect it from wind, rain and sun and to provide a **UV light** to increase its attractiveness to mosquitoes.

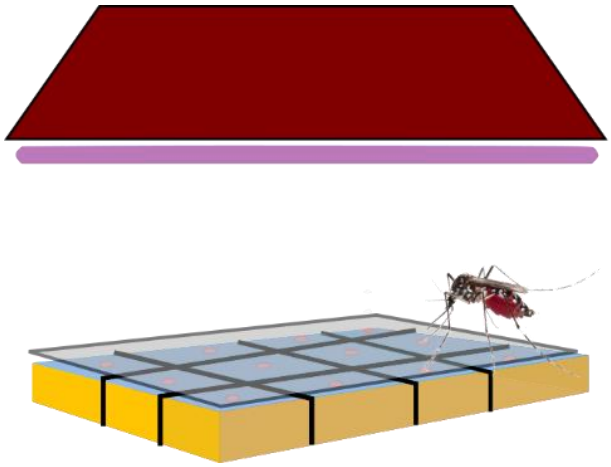

Figure 3.2.1: sample-collecting unit (simplified version, not taking into account the circuit and the refilling system).

3.2.2 Collecting the sample

To collect the samples automatically, we want to use a set of wells containing a sugar-rich solution, covered by the membrane; the wells are connected to a circuit that is activated when a well is bitten. To do so, each well has a **capacitance sensor**, with the two capacitive plates being positioned on top (the membrane) and on the bottom of the well. When the mosquito bites the membrane and injects its saliva in the well with its proboscis it activates the sensor ; then, the liquid from the well of interest - a mix of solution and **mosquito saliva** - is conveyed to the sequencing layer of the device (see **Section 3.3**) via a **syringe pump**[40], that must be positioned upstream of the system. To do this selectively, the system relies on a set of **electromechanical switches** (in red in the **Figure 3.1**). These can be on-off switches, that in resting position keep the tube compressed, rising when activated, leaving the solution with the mosquito saliva free to flow through the lumen of the tube. Ultimately, both the well and the syringe pump are automatically refilled with solution from the reservoir, also controlled by a switch.

To have a clearer description of the process, we explain each phase briefly, as shown in the **Figure 2.2**:

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[38] Van Breugel, F., Riffell, J., Fairhall, A., & Dickinson, M. H. (2015). Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets. Current Biology, 25(16), 2123–2129.doi:10.1016/j.cub.2015.06.046 Da citare per il fatto che sono attratti da odoranti

[39] https://mesamalaria.org/resource-hub/astmh-2016-felix-j-hol-interrogating-mosquito-pathogen-communities-using-high

[40] https://www.ossila.com/products/syringe-pump, http://www.syringepump.com/NE-1000.php referenze a possiblili syringe pumps

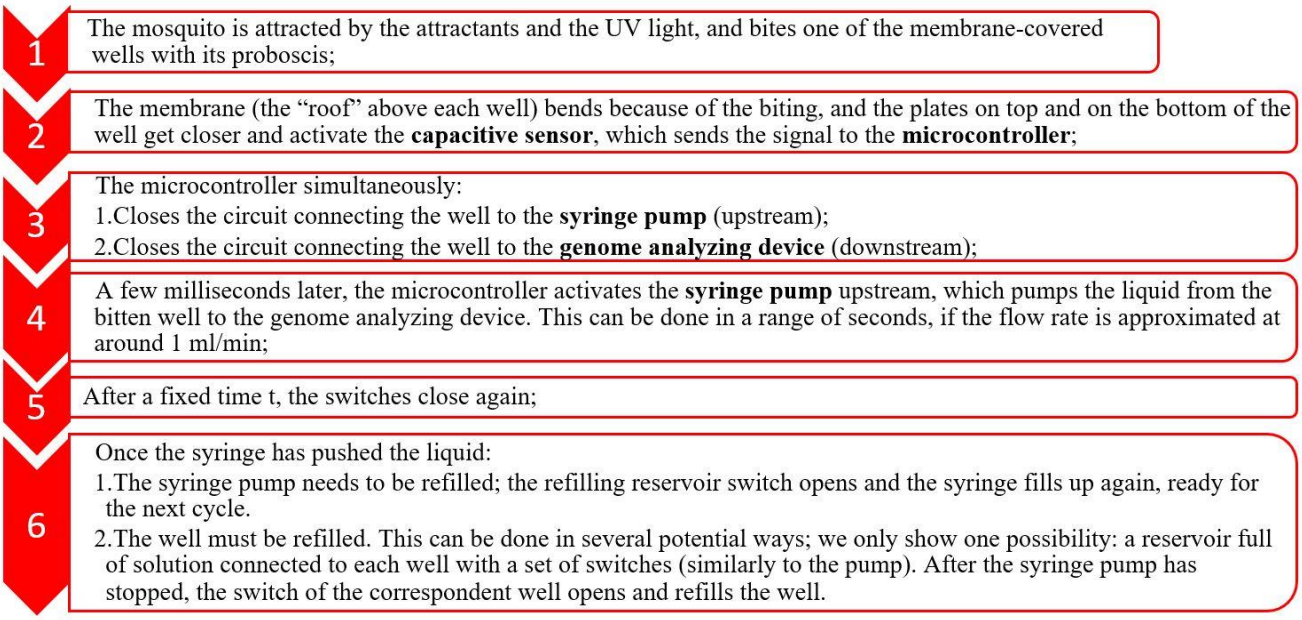

Figure 3.2.2: Sample collection process with GAMu.

Thanks to the microcontroller and the syringe pump, all the steps can be accurately timed, and the full collection process can take place in a range of **seconds**, triggered by a mosquito bite. Also, the system is not active when no insect bites and therefore the energy consumption is reduced.

To avoid that the same mosquito bites several times, the system can be programmed to stay **inactive** for some seconds after its activation. Also, it will be necessary to assess that there are no significant traces of the sample in the well after it has been "pumped" away.

3.3 Second layer: Oxford Nanopore Technologies

Once the biological sample has been pumped away from the well, it proceeds towards the technological tools needed to decipher the genetic information extracted from the vector.

Prior to sequencing, a selective amplification process increases the specificity of data sampling. In fact, mosquito bites may contain a huge variety of genetic material, either DNA or RNA-based, far beyond the set of viruses which are pathogenic to humans and therefore useful for our surveillance method. Some technologies have been recently developed for isothermic selective DNA amplification[41]. Arboviruses have RNA-based genomes, therefore an additional preparation phase should be performed on the sample through reverse transcriptase (RT) enzymes, commonly used in biomolecular lab procedures. Finally, the complementary DNA (cDNA) molecules obtained through RT application is the substrate of the sequencing device, which is the final destination of the biological sample collected, and the part of the process where biologically-encoded data is converted into electronic data, thus transmissible information.

The demanding requirements of this sequencing tool (automated, portable, near real-time) are met by a device developed by the Oxford Nanopore Technologies, MinION®[42]. It is based on the principle that the transit of linear molecules through a very small pore in a membrane must be orderly, reflecting the correct sequence of the molecule components. In the specific case, the linear molecule analyzed is a single-strand DNA segment, and nanopores are obtained by assembling protein multimers across a biological membrane. The only way DNA molecules can transit to the other side of the membrane is by passing through the pores in an orderly fashion. Sequencing is performed by measuring the electric signal generated by the transit of the DNA molecule through the pores, which is different for each of the four bases, as shown in **Figure 3.3.a**.

[41] Notomi, T., Mori, Y., Tomita, N., & Kanda, H. (2015). Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *Journal of Microbiology*, *53*(1), 1-5.
[42] https://nanoporetech.com/products/minion

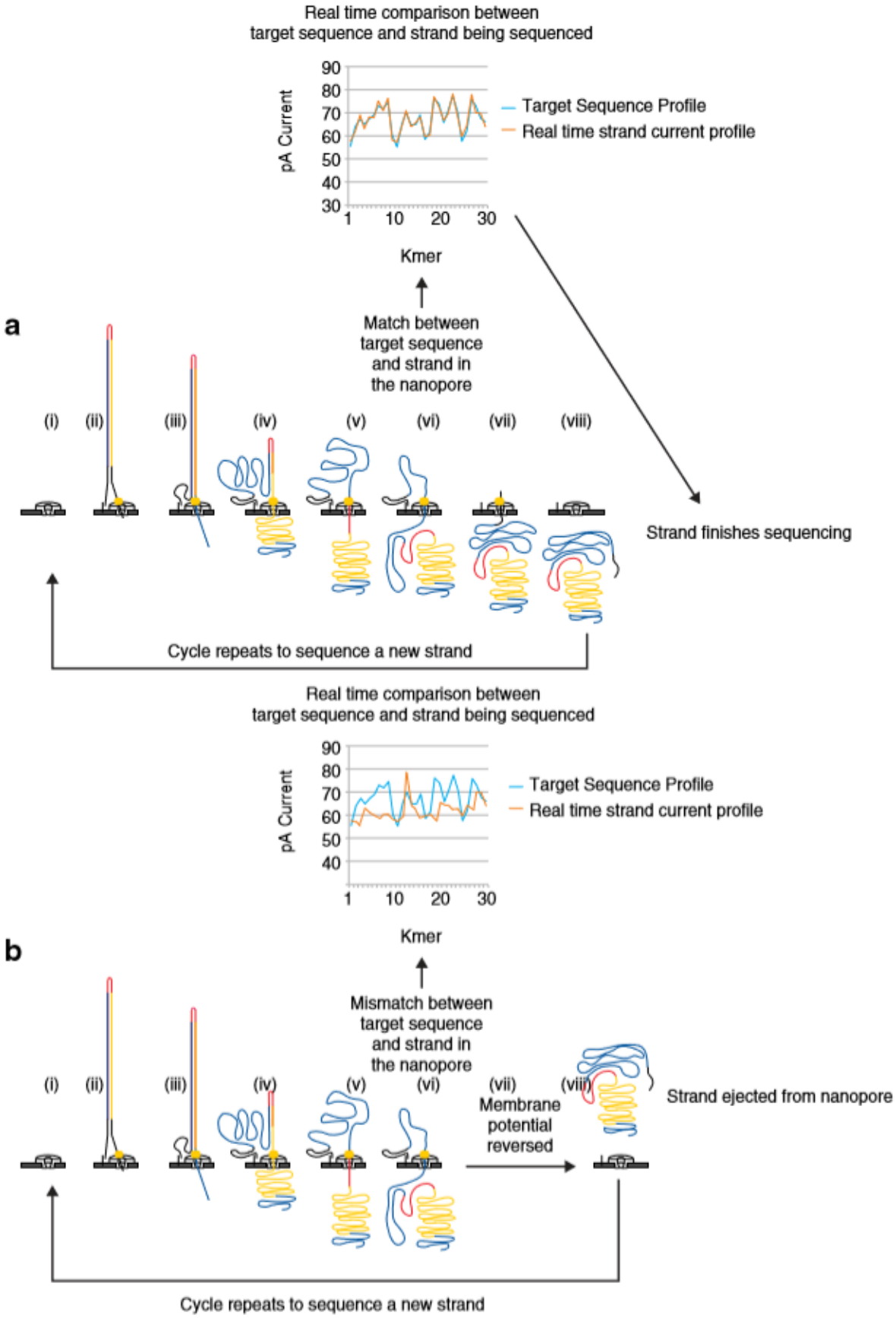

Figure 3.3.a: Functioning of the MinION technology. From **Jain et al, 2016**.[43]

[43] Jain, M., Olsen, H. E., Paten, B., & Akeson, M. (2016). The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome biology*, *17*(1), 239.

Prior to be sent to the MinION and after the amplification phase, the sample is pre-processed by the Voltrax®, an automated processing tool by Oxford nanopores that must be connected both to the DNA amplifier and onto the MinION (Oxford Nanopore has already declared they want to merge the two dispositives into one in the next future).

Figure 3.3.b: Structure of the Voltrax (on the left) and the MinION device (on the right)[44].

3.4 Third layer: data analysis, transmission and conservation

3.4.1 Data processing and targeted measures

Once the information about the presence of selected arboviruses is available from the MinION, it is handled in two different ways: the full sequencing information is locally **stored in an SSD** - that will be periodically checked during the maintenance procedures of the device - whereas a "**flag**" about the identified viral species is immediately sent via satellite.

This information will be part of a **network** (we refer to it as the Global Arbovirus Mapping Platform, but data might even be added to existing vector control databases) that will be accessed by different stakeholders and used for several measures, according to the context, as shown in **Table 3.4.1**

• **Urban areas:**
Transfusion and transplantation centers of local hospitals and clinics – they will strengthen control over donors as well as donated blood or organs;
Local health communication management center – will provide targeted hygiene and vaccination information to the area in which the device has detected the viruses;
Municipality – will proceed with targeted intervention through pest control and drainages in the selected area;
Local health agencies – they will get the complete sequencing data monthly, during the maintenance procedures the device. They will then adjust the targeting strategy to the specific viroma in order to obtain improved results.
• **Rural areas**:
Local health centers – will help with the maintenance of the device once every month and get the SSD storage card for analysis. Together with the other actors, it will get the relevant information on pathogens of interest and identify the best strategies of intervention accordingly;
National health authorities – will contribute to the development of the rural area strategy and contact the relevant stakeholders;
WHO - will support ad hoc measures to fight vector spread in those areas.
• **Scientific purposes**
Scientists and researchers – will have access to the data that will help implementing new mathematical models of viral diffusion, (see **Paragraph 3.4.2**).

Table 3.4.1: Potential users of GAMs data.

[44] https://nanoporetech.com/products/minion

3.4.2 Mapping Platform and Data Elaboration – Monitoring vectors and people

The information will be sent to the Global Arboviruses Mapping Platform (or to another network), which will collect the data and update the platform, consenting to monitor and predict virus spreading.

Epidemiological models are available within **Network theory**: models such as SI, SIS, SIR are classic instruments to understand the spread of habits or diseases within a network of known structure[45][46]. In our case nodes of the network could be represented by mosquitoes, people or a weighted network with nodes representing an area, their dimension informing on the density of specimens in that area.

We envision the adoption of predictive models such as GLEAM: the power of our device can be fully harnessed with a model that can help foresee the real time spread of a pathogen and **predict** the number of infected individuals expected each week both in urban and in rural areas. Pandemic forecasting has already been successfully deployed thanks to models like GLEAM (H1N1 prediction in 2009[47]). The model requires demographic data, mobility data and models the spread of a virus among a population. It can consider possible factors and model different scenarios, based on the **aggressiveness of the virus and on possible response strategies that can be employed.** A more detailed flowchart is provided in **Figure 3.4.2**.

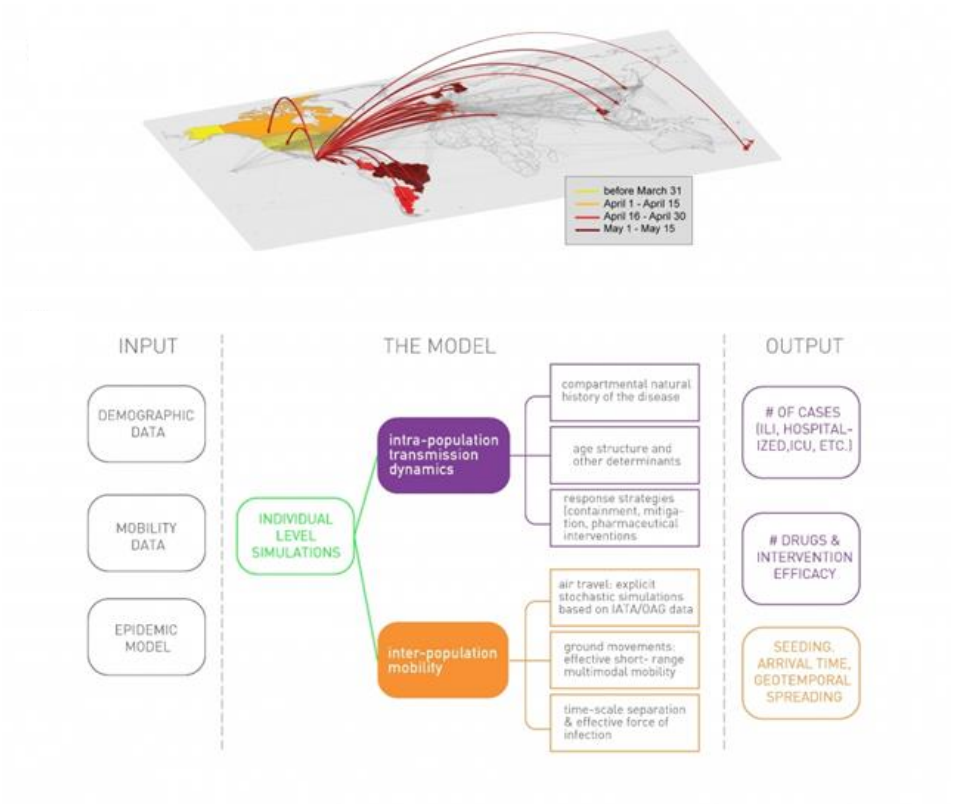

Figure 3.4.2: GLEAM model flowchart. From **Tizzoni et al, 2009**.[48]

Since the model has been crafted by Northeastern University, thinking of a spread among humans and not vectors, we would have to tweak it, integrating entomological data on the presence of mosquitoes in some areas, and looking at the traveling radius of mosquitoes.

Given that GLEAM has been already deployed taking into consideration spread among humans, a possible extension could encompass the **integration of human data coming from the different stakeholders**: an integration of human-vector data, other than enlarging the dataset, helps driving up precision since it adapts to what the models already ask for.

[45] R. Pastor-Satorras and A. Vespignani. Epidemic spreading in scale free networks. Physical Review Letters, 86:3200–3203, 2001.
[46] R.M. Anderson and R.M. May. Infectious Diseases of Humans: Dynamics and Control. Oxford University Press, Oxford, 1992.
[47] M. Tizzoni, P. Bajardi, C. Poletto, J. J. Ramasco, D. Balcan, B. Gonçalves, N. Perra, V. Colizza, and A. Vespignani. Real-time numerical forecast of global epidemic spreading: case study of 2009 A/H1N1pdm.
[48] *Ibidem.*

3.5 Expected costs

The technologies used for GAMu are at the frontiers of innovation and technological development. Given this fact, the device is substantially more expensive than other traditional mosquito traps, but far less than mobile laboratories, such as the ones used by the WHO to early diagnose Yellow Fever in Democratic Republic of the Congo[49].

The innovative structure of GAMu and its complexity do not allow for a precise estimate of the cost of all specific components; therefore, we estimated a possible comprehensive price, considering market prices for single parts and excluding possible discounts, coherently with a cautious approach. In the **Table 3.5**, expected costs for GAMu are presented:

Layer	Object	Expected cost	Energy consume
Outdoor	Roof and UV light	$ 20	15.000 mAh/day
First	Parafilm with chemioattractans, circuit, capacitance sensor, microcontroller, electromechanical switches	$ 20	100 mAh/day
First	Syringe pump	$ 750	300 mAh/day
Second	VolTrax	$ 8.150	3.000 mAh/day
Second	MinION	$ 1.000	1.000 mA/day
Third	SSD (1T) and data transmitter	$ 600	2.000 mA/day
Energy	Solar panel and high-capacity rechargeable 30.000 mAh battery.	$ 300	
Total	**GAMu**	**$ 10.840**	**21400 mA/day**

Table 3.5: expected cost and energy budget for one GAMu.

The total cost of $ 10.840 is an *a priori* budget, which will be re-assessed during the prototyping phase, that will have a crucial role in determining the exact acquisition cost and implementation cost of each component. Agreements with suppliers and elimination of all the superfluous functions of the Oxford Nanopore technologies will be drivers for cost reduction. Moreover, the prototyping phase will allow us to precisely identify energy consumes. For the moment we estimated an energy budget, calculated in a joint evaluation between the group of Authors and senior Engineers with expertise in automated mechanical systems. These numbers are not negligible, but, considering the possible benefits of GAMs, this investment could be cost-effective. Anticipating the detection of areas at a high risk of a specific mosquito borne disease through vector monitoring and controlling can lead to preventive interventions, vaccines, hygienic improvements, and population health education.

A study carried out in Brazil[50] showed that an Intelligent Dengue Monitoring System, composed of traditional mosquito traps checked periodically, a data sharing platform and virus genome analysis, prevented 27,191 cases of dengue fever and saved an average of $227 (median $58) per case prevented, which saved approximately $364,517 in direct costs (health care and vector control) and $7,138,940 in lost wages (societal effect) annually. Considering that our system will be able to track non only a specific virus but a variety of viruses, and that the information collected by our device are much more precise and detailed, we could imagine even a greater impact on cases prevention and timely detection of possible outbreaks.

Further analysis of cost-effectiveness will have to be carried out, once the definitive costs are assessed; in the meanwhile, partnerships with financial stakeholders, such as the Bill and Melinda Gates Foundation, or the Wellcome Trust Innovator Award, will be crucial in collecting the necessary investments for the prototyping phase.

[49] https://www.who.int/news-room/feature-stories/detail/mobile-labs-deliver-faster-yellow-fever-test-results
[50] Pepin, K. M., Marques-Toledo, C., Scherer, L., Morais, M. M., Ellis, B., & Eiras, A. E. (2013). Cost-effectiveness of novel system of mosquito surveillance and control, Brazil. Emerging infectious diseases, 19(4), 542.

4. Project implementation

4.1. Geographical application

Our project will see its first application in Brazil, in the metropolitan area of **Rio de Janeiro**, in particular, we propose the **northern quartier of Manguinhos** (**Figure 4.1.a**). This is for several reasons.

First of all, Brazil, according to the Pan American Health Organization and the WHO, sees the presence of 11 vector borne diseases (Dengue, Chikungunya, Zika, Malaria, Chagas, Leishmaniasis, Yellow Fever, Onchocerciasis, Plague, Lymphatic Filariasis, Schistosomiasis)[51], making the detection of arboviruses and their eventual mutations highly probable.
Secondly, the city of Rio de Janeiro is the second largest urban center in Brazil, and, as recognized by the Arbovirus Surveillance Program of the Municipal Health Department, has had five major Dengue epidemics since 1986[52]. Moreover, in 2015 Zika and Chikungunya viruses have been introduced, causing sequential epidemics in 2015 and 2016[53].
Thirdly, the growth of slums and irregular constructions in overcrowded urban centers, combined with the poor sanitation levels and the inefficient garbage collection favored the reproduction of *Aedes aegypti* mosquito, making it very difficult to control vector borne diseases.
Fourthly, the outer urban areas of Rio de Janeiro, such as the Manguinhos bairro, are especially prone to *Aedes aegypti* infestation because of lack of water supply and, as said, bad garbage collection system, resulting in favorable breeding sites for arboviruses vectors[54].
Fifthly, through the Italian-Brazilian lab for training, research and practices in collective health, situated in Bologna[55], we can enter in direct contact with the main health stakeholders of the city of Rio de Janeiro.
Sixthly, the city has already a strong arbovirus detection system, however mostly based on post-infection detection. Taking advantage of the information already available through hospitals and the Arbovirus Surveillance Program of the Municipal Health Department, it will be possible to combine it with our device's results and create a real-time mapping system of the city's most vulnerable quartiers and prevent new infections through targeted measures.
Finally, Brazil and the city of Rio de Janeiro are strongly affected by Dengue, considered to be one of the most crucial ten global health threats of 2019 by the WHO[56].

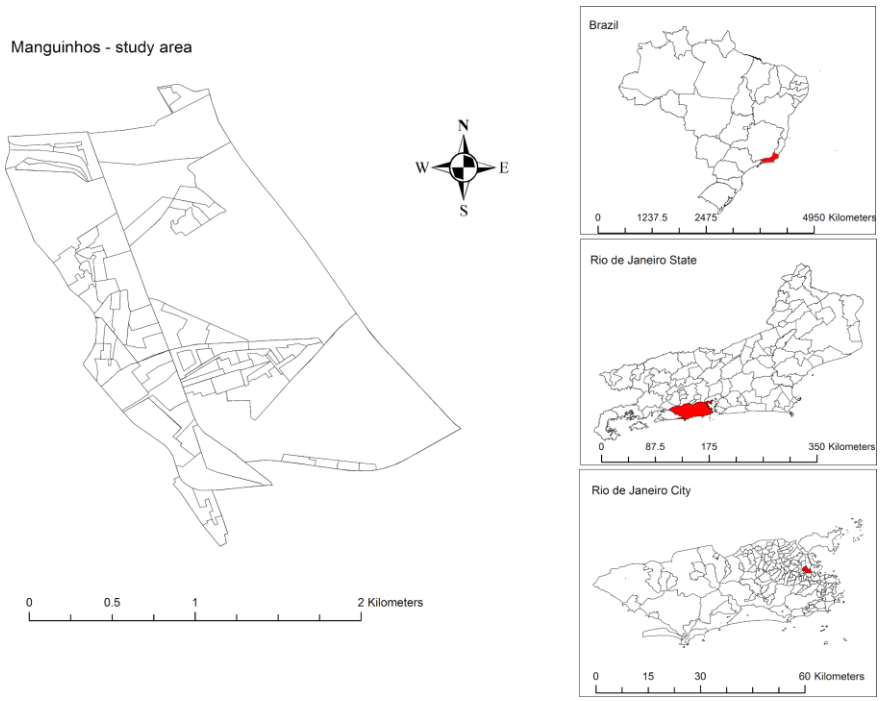

Figure 4.1.a: Map of Manguinhos bairro, Rio de Janeiro.[57]

[51] PAHO, Vector Borne Diseases (http://ais.paho.org/phip/viz/cha_cd_vectorborndiseases.asp)
[52] Rodrigues NCP, Daumas RP, de Almeida AS, dos Santos RS, Koster I, Rodrigues PP, et al. (2018) Risk factors for arbovirus infections in a low-income community of Rio de Janeiro, Brazil, 2015-2016. PLoS ONE 13(6): e0198357.
[53] *Ibidem.*
[54] *Ibidem.*
[55] https://site.unibo.it/almaengage/en/projects/italian-brazilian-lab-for-training-research-and-practices-in-collective-health
[56] WHO, Ten threats to global health in 2019 (https://www.who.int/emergencies/ten-threats-to-global-health-in-2019)
[57] Rodrigues, N. C. P., Daumas, R. P., de Almeida, A. S., dos Santos, R. S., Koster, I., Rodrigues, P. P., ... & da Costa Leite, I. (2018). Risk factors for arbovirus infections in a low-income community of Rio de Janeiro, Brazil, 2015-2016. *PloS one*, *13*(6).

During the pilot project, we will test the device through a practical application, in order to develop a cost-benefit analysis and assess the functioning of the data flow to the central sanitation system. This will allow us to precisely estimate the costs of the implementation of our device, the necessary policy framework needed in order to best exploit the real-time data inflow in order to deploy *ad hoc* measures, and the configuration of the Global Arbovirus Mapping Platform.

4.2. Project implementation strategy

The Pilot Project is divided into four steps: creating partnerships, prototype development, validation & scaling up, and finally operation & maintenance. These phases are logically subsequent, but some of them will be implemented simultaneously, as shown in Paragraph 4.3. To be more specific, our main macro-actions for the pilot project will be:

Creating partnerships: The partnerships will be divided in technical, financial, governmental, and local community partners. We are planning on developing a direct connection with the various stakeholders in the city of Rio de Janeiro, especially through the Italian-Brazilian lab for training, research and practices in collective health[58], in order to start enquiring on the possibility to create a center for the elaboration of the incoming data flow from our devices. Our potential partners are shown in **Annex II**.

Prototype development: Our team will seek a collaborative technical partnership with two avant-garde departments of the Scuola Superiore Sant'Anna, the Biorobotics Institute and the Technology of Information, Communication and Perception Institute (TeCIP), in order to develop the prototype, in partnership with Oxford Nanopore Technologies and the VectorChip Team of Stanford University. Moreover, TeCIP Institute will an important partner for the creation of the GAM Platform.

Prototype validation & scale-up: The prototype will be tested in the Barrio of Manguinhos for a period of 15 months, starting February 2021[59] in order to define its cost-effectiveness and policy implementation. It will be placed at a distance of ca. 1 km around the perimeter of the barrio and inside the nucleus. It will be slightly overhead and adjusted to the specific meteorological conditions of the position. The data will be sent to the Municipal Health Center João Barros Barreto, which will inform the Municipality of the city of Rio de Janeiro and the Health Department of the state of Rio de Janeiro. The latter, in coordination with the Municipal Health center, will deploy targeted measures such as pest control and drainage, as well as hygiene actions in order to reduce the incidence of the virus found. The Municipal Health Center will also supervise the effective update of the GAM Platform,and provide information about the measures and their effectiveness.
After this phase, the scaling-up of the project will start from Italy, having found interest from the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale" in applying the device to the Italian context. This new placement of the device will depend on the Institute's estimates on June 2022 mosquito hotspots. The Institute, being party in the European Disease Control Center, will than contribute with the scaling-up of the device and the Mapping Platform at European level, following climatic hotspots and migration flows. Finally, the project will be implemented in rural and urban areas in the African continent first, in particular Tunisia and Namibia, with which the Institute has ongoing partnerships, and Ethiopia, with which the Scuola Superiore Sant'Anna has tight contacts.

Operation & maintenance: The data on the project's operationality will be collected on a daily basis, analysed by the Municipal Health Center João Barros Barreto and confronted with local hospital data as well as from the local arbovirus monitoring system. Human-based interventions will be taken into consideration and the prototype will be modified accordingly to the needs observed by the on-site personnel. Moreover, the policy requirements will be evaluated, and the most effective policy processing system will be developed in order to guarantee structured, immediate and targeted intervention in vulnerable areas.

4.3. Timeline

Given the technological complexity of GAMu, an extensive prototype development is necessary. As shown in **Table 4.3**, after the ideation and the expert phase, a 16 months period dedicated to prototyping has been considered. During this phase, collaborations with the BioRobotics Institute Sant'Anna, the TeCIP Institute Sant'Anna, Oxford Nanopore Technologies, IZT, and Stanford University VectorChip team will be crucial. Moreover, this phase will be essential in order to assess the feasibility, costs, and the potential of our device.

[58] https://site.unibo.it/almaengage/en/projects/italian-brazilian-lab-for-training-research-and-practices-in-collective-health
[59] Lana et al. demonstrate that the period summer and fall months (January to May) has overall the highest mosquito abundance rate, Lana et al., Seasonal and nonseasonal dynamics of Aedes aegypti in Rio de Janeiro, Brazil: Fitting mathematical models to trap data, Acta Tropica 129 (2014) 25–32, Elsevier, 2013.

Field implementations will start in February 2021 with the pilot project in Manguinhos Barrio in Rio de Janeiro. If validation results and cost-benefit analysis over a period of 15 months prove to be sufficiently positive, the scaling-up of the GAMs will take place, starting in May/June 2022 in Italy and expand further from October 2022.

	Mar Apr 19	May Jun 19	Jul Aug 19	Sep Oct 19	Nov Dec 19	Jan Feb 20	Mar Apr 20	May Jun 20	Jul Aug 20	Sep Oct 20	Nov Dec 20	Jan Feb 21	Mar Apr 21	May Jun 21	Jul Aug 21	Sep Oct 21	Nov Dec 21	Jan Feb 22	Mar Apr 22	May Jun 22	Jul Aug 22	Sep Oct 22	Nov Dec 22
Ideation phase (research and development of the idea)																							
Expert Phase (Consultation with various experts from the field)																							
Prototype designing and building (preliminary study, components assemblage, software development)																							
Partnership development (Technical, financial and governmental and local partnership)				Pilot	Pilot	Pilot				Pilot	Pilot	Pilot						Italy	Italy	Italy	Scaling up	Scaling up	Scaling up
Pilot Project (Testing in Manguinhos Barrio Rio de Janeiro)																							
Application in Italy (Testing the device on the European coast)																							
Initiating the Scaling-up process (Testing the device in different contexts - Europe, Tunisia, Ethiopia, Namibia)																							

Table 4.3: Estimated timeline for the GAMu prototyping and the GAMs implementation.

During the **expert phase**, we received useful and insightful suggestions from many professors and researchers, cited in the **Annex III.**

4.4. Stakeholder analysis

Table 4.4. shows the major stakeholders for the implementation in Manguinhos Barrio in Rio de Janeiro:

Category	Stakeholder/entity	Role in the project
Beneficiaries	○ Inhabitants of the Manguinhos barrio, especially pregnant women and children ○ Urban health agencies and hospitals	Benefit from: ○ Timely information on virus presence ○ Improvement in health intervention ○ Better health and less cases of VBD ○ More economic and social development
Project clients	○ Municipal Health Center João Barros Barreto ○ Health Department of the State of Rio de Janeiro ○ Ministry of Health of Brazil ○ WHO ○ Pan-American Health Organization	○ Strategic partnerships for pilot project implementation ○ Use of GAMs data to plan preventive interventions and built computational model to predict vector dynamics
Financial partners	○ Bill and Melinda Gates Foundation ○ Wellcome Trust Innovator Award ○ Foundation for NIH ○ World Bank ○ UN Foundation	○ Financial support in GAMu prototyping and pilot project implementation
Technical partners	○ Sant'Anna Biorobotics Institute ○ Sant'Anna TeCip Institute ○ Oxford Nanopore Technologies ○ VectorChip Team of Stanford University	○ Technical support in the development and prototyping of GAMu and GAMs
Allies	○ NGO Associação Saúde da Família (ASF) ○ Independent social organization Associação Saúde Criança (ASC)	○ Contributions to our mission thanks to synergies and common objectives ○ Link to local communities and local human resources ○ Sharing of their network

Table 4.4: Stakeholder analysis for the pilot project in Rio de Janeiro.

An estimate of the project budget and risks analysis are presented in **Annex IV** and **Annex V**.

5. Limitations

Given several technological and methodological limitations, we are aware that The Global Arbovirus Mapping System presents a number of limitations which have to be addressed:

The device

As we have presented it in its different components, which are based on high-technology tools, our GAMu exceeds the **costs** of most surveillance strategies based on traditional vector sampling and manual analysis. Nonetheless, we believe that the potential advantages deriving from automated, real-time epidemiological and microbiological information about single virus variants is both revolutionary in the study of virus strains fitness and spread capabilities, and priceless in the future, in case of pandemic vector-borne diseases which will need to be stopped immediately through efficient and global-scaled methods of prevention. Also, the speed of technological advances is usually coupled by a significant reduction in costs: new, cheaper ways to develop the same technologies are often found quickly after their development. Therefore, we hope that in the next future the cost of the sample analyzing devices will decrease.

Another potential limitation derives from the **power budget analysis** of our devices, which must take into account not only the intrinsic device functioning, but also the data transmission systems. A possible solution to this issue is increasing the capacity of its battery, which would not impact significantly on the cost of the GAMu. Also, the association with solar panels can increase the autonomy of lithium rechargeable batteries.

Another concern is represented by the issues connected with the **spatial collocation** of the device. Both rural and urban contexts present risks for the integrity of our high-technology devices, because of animal attacks, climatic phenomena, human manumission. However, simple design features such as solid roof and enclosures should minimize these risks for our GAMus.

A further problem is represented by the **timing** of the sequencing that, depending on the length of the sequences amplified, could require a time scale which exceeds the hour. However, this can be overcome by reducing amplification before sequencing, with the tradeoff of a lower base coverage, that can be accepted considering the high length of the sequences that can be analyzed with the Oxford technology.

An intrinsic limitation regarding the metrics of the stations functioning is **maintenance**: all the high-technology components which have already been proven to work singularly and in laboratory or in wildlife context, are here required to function together, in an unsupervised way. Limiting factors in this process are represented by **reagent stability** and periodic provision, as well as the reasonable risk of structural damage due to animal attack or atmospheric agents (even with an aluminum cage surrounding the device), needing human intervention.

Also, the **handling of reagents**, including their discarding and recharging after each sample has been processed, must be performed automatically. Nevertheless, we think that this process can be automated in a relatively easy way, and plan to ask for further consultation to have a clearer idea about its implementation. Periodic **maintenance** should be taken into account for each of our devices, which increases the functioning costs and reduces the comparative advantage towards the totally supervised surveillance methods that already exist. Nevertheless, we think that this reduction would not be significant if we reached the reasonable goal of a **monthly** frequency of human intervention. However, considering the lack of similar devices on the market at the moment, we would need to try a prototype to validate our esteem and make the necessary corrections.

Methodology

Methodological limitations to the project we propose are represented by all the factors which reduce the sensitivity and specificity of our data collection.

Sensitivity is reduced by procedural errors that prevent useful genomic information to be collected or transmitted, such as the lack of attractiveness our device has towards mosquitoes, possible positive or negative biases regarding vector species, issues in genetic material amplification or sequencing, and errors in data transmission. In the light of this brief analysis, maximization of sensitivity is possible with the implementation of single components with optimal functioning in unsupervised settings. Apart from the use of microcontroller systems like the one we have described in the first layer of the device, no disruptive change in current technology is required for reaching this aim.

Reduction in **specificity** occurs for every reason that leads to the collection of useless information, different from the detection and characterization of the vector virome. This is likely to happen with genetic material belonging to the mosquito itself and to viruses which are not pathogenic for humans. Moreover, mosquito populations often have very **low carriage** rates, whereby only one in 1000 mosquitoes are infected.

To increase the probability of detection, large numbers of mosquitoes are required, resulting in numerous mosquitoes to identify, pool and test, increasing turnaround time. In maximizing the specificity of data collection, a first useful item is represented by the system of biological interaction with the vector: our choice of not killing the mosquito, but taking the saliva it regurgitates through the biting process, is focused to reproduce as much as possible the **biological interaction** with humans, including all and only the molecules the vector would inject in a bite. Also, the optimal choice of chemoattractant could increase the species-specificity of the vector-device interaction.

A further way of increasing specificity in genetic sequencing is **selectively amplifying** the biological sample with primers based on **conserved genetic regions** of the different virus families or genera. Thus, the sequencing process would be specifically focused towards detection and monitoring of single species or variants of interest. In this context, detection of new viral strains would simply result from the sequencing process, without loss of sensitivity. Also, depending on the primer specificity and the potentialities of the Oxford nanopore technology, we could try to sequence in parallel the mosquito DNA and the viral cDNA and stop the process if no information of interest is extracted. Indeed, using primers both for conserved regions of mosquitoes and viral species, we could selectively program device to stop the sequencing if any viral cDNA is not detected, only reporting a flag that a species of mosquitoes has been found without carrying a virus.

Applications

In the final stage of our project, a large amount of data will continuously flow in near real-time (depending on the sequencing time, as shown in the previous paragraph) into globally-accessible databases, allowing the prompt attuation of strategies aimed to contain the viral spread. Of course, this process is limited by the effectiveness of **data elaboration** and, especially, of the **policy answer** to the precious information extracted from the database. Shared surveillance and intervention protocols should be agreed by health authorities of different States at a global scale, possibly under the egida of the World Health Organization.

6. Future applications and conclusions

The Global Arbovirus Mapping system could give a decisive contribution to strengthening the preparedness and response actions, as well as the capacity for early detection, confirmation and surveillance of emerging and mosquito-borne diseases at the global level by providing local, national and global health agencies and stakeholders with near real-time epidemiological data and relevant information on vectors in hotspot areas.

What we really believe to be unique and innovative in this idea is its incredible scalability, intended both in a *spatial* and above all in a *viral* sense: choosing the right amplification primers, in principle it would be possible to obtain data about an enormous pool of pathogens of interests in a real-time context, also including non-viral pathogens such as malaria-carrying *plasmodium.* This would also justify the high costs of the device.

For the aforementioned reasons, a possible future application of the device could be in Mediterranean coasts that are becoming increasingly interested by new vectors, owing to climate changes. Especially in this context, an "unprejudiced" device, that can potentially identify several different viruses and their carrying vectors could prove a useful resource for early detection and intervention measures.

Also, the potential benefit of a similar device for mathematical modeling is enormous: considering that many of the actual maps and models of viral epidemics rely on data about infected people, GAMs would help building a more precise picture.

Overall, even if the device would prove too costly for a large-scale implementation, it would still be of great interest for research purposes, allowing to position a single GAMu in an isolated, low resource area of interest to collect data for a prolonged period, which could be accessed remotely from the laboratory.

Despite their young age, the authors of this work have been scientifically ambitious and admittedly provocative, while honestly believing in the potentiality and feasibility of the device, even considering its technical difficulties. Therefore, they will try to get funding in the next future to build a prototype and hope that their work will make a real contribution to the surveillance methods of mosquito-borne diseases surveillance.

"The one thing I would change about the world is to transform my colleagues in academia to kids all over again, so they would follow the sincere path of learning about the world."
Avi Loeb

"I wish Noah would have swatted the mosquitoes."
Anonymous

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Annexes

Annex I – Current Surveillance Methods

WHAT IS BEING USED	***How***	***Where***	***Benefits***	***Problematics***
Human or animal case surveillance	Healthcare providers report suspected cases and submit blood samples[60].	Asia-Pacific, America Africa	Captures spatiotemporal patterns of variation in incidence. Allows characterization of viruses	Costly, Suboptimal efficiency
Sentinel animals	Assessment of serum samples from primates for arbovirus-specific antibodies.[61]	Mato Grosso do Sul (Brazil)	Feasibility Information about viruses in the natural environment	Difficult systematization Low samples numerosity Ethical implications of involving non-human primates Cross-reactivity among related viruses
Human vaccination	Active immunization of susceptible humans	Endemic areas for YFV and DENV transmission	Effective in preventing transmission Risk-free Cost-effective	Human vaccines only available for Yellow Fever Virus and, recently, for Dengue Virus Herd immunity affected by variable adherence in susceptible populations
Infection prevention of transmitting vector (Transmission-Blocking Vaccines)	Prevention of mosquito midgut cells infection through human "altruistic" vaccination[62].			Risk of cross-reactions or autoimmune disease in humans Treated humans may not be immunized towards the virus High titers of infection-blocking antibodies required in vaccinated individuals
Genetic modification of vectors	Gene drive "population replacement" in which a mosquito population biologically able to transmit pathogens is "replaced" by one that is unable to transmit pathogens. "Population suppression" through release in the environment of genetically modified sterile mosquitoes[63].	Globally	No risk of infection from male genetically modified mosquito	Need for continuous reintroduction of genetically modified mosquitoes. Gene drive "population replacement" might not work as intended and could increase the prevalence of some types of mosquito-borne diseases.
Vector biological control and environmental management	Insecticide treatment of indoor and outdoor spaces Mechanical barriers to limiting vector-host contact (e.g. bed nets) Reduction of breeding sites	Globally	Direct confrontation with the problem	Insecticide resistance
BG-Counter	Automatically differentiates mosquitoes from other insects entering the trap, counts them, and wirelessly transmits the results to a cloud server.	Commercially available	Via a web application you can manage your mosquito traps and get new insights into daily activity patterns, adult density indices, population dynamics and effectiveness of your control activities	Only counting mosquitoes

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PILOTS	***How***	***Where***	***Benefits***	***Problematics***
BG-Sentinel traps (Unbaited, with CO2, with CO2 and 1-octen-3-ol)	A white cylindrical container with dry ice covered with gauze in which ascending currents of the attractant are generated in the center of the trap, where there is a catch bag and a fan that sucks up the mosquitoes. Octenol is a chemical contained in human breath and sweat whereas BG-Lure contains a combination of substances found on human skin (lactic acid, ammonia, and fatty acids[64]	Southern Europe, Western Australia	BG-Sentinel traps with attractants and CO2 are significantly better at capturing mosquitoes that have fed on mammals than CDC-CO2 traps in the cases of An. atroparvus and Cx. theileri. BG-Sentinel traps that use CO2 and attractants are as effective as CDC-CO2 traps for Culex mosquito species, Ochlerotatus caspius, and they are also highly efficient at capturing Anopheles atroparvus host-seeking and blood-fed females with or without CO2.	Maintenance of a cold chain through the use of dry ice or liquid nitrogen ship-pers in the field required during the transportation from the trap to the laboratory, which can be logistically challenging. Virus isolation is time consuming and obtaining definitive results can take weeks, which defeats the purpose of using it for early warning BGS traps with octenol and CO2 don't attract significantly more mammal-fed mosquitoes than BGS traps with BG-lure and CO2. No significant differences in the relative abundance of host-seeking female mosquitoes trapped in CDC-CO2 and BGS traps with CO2 and attractants
CDC mosquito traps with CO2	The CO2 attracts mosquitoes, which are then sucked up with a fan[65].	Southern Europe, Western Australia		Dry ice problems and time consuming procedure. The CDC-CO2 trap is the least efficient method for capturing blood-fed females
Excreta collection mosquito trap	Using room-temperature "nucleic acid preservation cards" left in baited traps, the excreta is collected by adding a polycarbonate collection sheet that is manually wiped with one of the nucleic acid preservation cards, or by placing one of those cards where the insects relieved themselves onto it directly (van den Hunk et al., 2019)	Western Australia		
Microsoft's Project Premonition mosquito trap	The system is designed to recognize which mosquito it is supposed to catch based on the flap of the mosquito's wing	Houston	The trap can tell what time each mosquito was trapped, as well as what the temperature, wind and humidity was when the mosquito flew in, and it's designed to withstand the rain, wind and other elements.	Still in testing phase, captures bugs in general.
VectorChip Stanford University	Large-scale autonomous collection of individual saliva droplets originating from single mosquito bites that can be used to identify the mosquito and pathogen species, including a broad range of human biting mosquitoes and numerous pathogens[66].	Stanford	Low cost of sample collection and analyses allows for high spatiotemporal resolution monitoring of pathogen occurrence in mosquitoes	Analysis in laboratory Need to empty droplets contained and analyse them in a laboratory

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Annex II – Potential partners

Organization	Role
The Arbovirus Surveillance Program of the Municipal Health Department of Rio de Janeiro,	Local supervisor;
The Pan-American Health Organization,	Coordinator of the project at South American level;
The Stanford University VectorChip team	Application of their VectorChip and Membrane on our device;
Oxford Nanopore Technologies	Private organization working on the incorporation of the needed technology;
The Brazilian Ecovec company,	To implement our project in collaboration with their MosquiTrap in Rio de Janeiro;
The Bill and Melinda Gates Foundation, among others,	For future financing of the project;
The Emerging Viral Diseases-Expert Laboratory Network and VectorNet	For the integration of our device in the European network for a comprehensive data flow (second pilot project);
The Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "Giuseppe Caporale",	Has shown interest in collaborating with the team in order to integrate the device and Platform within the Italian and European context.

Annex III– Contributions

Name	Contribution area
Prof. Peter Hudson, PennState University, Huck Institute Director	Biological components and potentialities of our device;
Prof. Francesca Chiaromonte, statistician at **Scuola Superiore Sant'Anna and PennState University**	Data analysis, statistical reasoning and implementation phase;
Prof. Leonardo Ricotti, BioEngineering and BioRobotics professor at **Scuola Superiore Sant'Anna**	Device construction and feasibility;
Prof. Tommaso Cucinotta, associate professor at the Real-Time Systems Laboratory within the **TeCIP Institute, Scuola Superiore Sant'Anna**	Energy-related needs and data transfer.
Doctor Davide Mei, Neurology and Genetics researcher at the **Pediatric Hospital Meyer in Florence**	Need assessment of the device and the technological framework of it
Prof. Quique Bassat, Pediatrician, researcher at **ISGlobal** and **ICREA** research professor	Critical aspects of on-field implementation;
Prof. Mauro Pistello, Complex Universitary Virology Operative Unit, **University of Pisa**	Scientific and clinical treatment of arboviral diseases.
Doctor Giovanni Savini and **Maria Goffredo**, Experimental Zooprophylactic **Institute of Abruzzo and Molise** (IZS)	Insights on mosquito vital cycle, spread and virological charge.
Prof. Lara Tavoschi, Department of Translational Research on New Technologies in Medicine and Surgery **and European Center for Disease Control**	impact-driven applications of GAMs and policy framework.
Researcher Sara Mazzilli, PhD candidate at **Università di Pisa**	Hygiene and related political framework.

Annex IV– Project Budgeting

The project costs will be defined during the prototyping phase, since it is necessary to first assess the technical capabilities and cost of our device. The main area of cost to be considered are the ones shown in the following Table:

Macro category	Drivers of cost
Cost of prototype	○ Device costs ○ Software development and Global Arbovirus Mapping Platform installation ○ Labor costs
Cost of maintenance	○ Training of the labor force ○ Organic material for CPR and GAMu functioning ○ Labor costs for regular maintenance
Cost of policy implementation	○ Pest Control ○ Vaccination ○ Drainage ○ Information and hygiene campaigns

Annex V– Risks analysis

During the prototyping phase, a more detailed risk analysis will have to be performed. Just to give examples of possible risks, we present in the following Table some of the implications that will have to be considered and analysed before the implementation project.

RISK	IMPLICATIONS
Harming of the device	○ Risk of thieves in urban context ○ Risk of animals in rural context ○ Risk of extreme weather events everywhere
Breaking of the device	○ Risk of breakage of the device's components ○ Risk of inability to process and transmit information Risk of power shortages
Data collection problems	○ Risk of ineffectiveness of timely data detection
Policy problems	○ Risk of tardiness in deploying targeted measures