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Paulo Jorge **Nogueira** *e d i t o r i a l*

Thematic number: Dengue in Madeira

When we published the first number of this journal - Health by Numbers Portugal – we thought about making a special issue (a thematic issue). The occurrence of Dengue outbreak in Madeira was then very recent, proposing itself naturally as an attractive and current theme.

Despite the time lag that has elapsed, it is particularly gratifying that we now have a record of what happened, what was exemplary done, and we can point out what could possibly have been done additionally. This is an account of some health professionals who have been directly involved in the outbreak; representing the very many who were in fact "on the field" in the Autonomous Region of Madeira or backing them.

By coincidence we are currently witnessing an Ebola outbreak in Africa that is driving renewed reflection on existing plans and promoting respective updates; in Portugal we just had a Legionella outbreak followed with interest either in Portugal and outside its borders; I.e., there is work, experience and new knowledge that is being generated that will hopefully follow the steps of this thematic issue and end up coming here.

With regard to this number two dedicated to the Dengue event occurred in the Autonomous Region of Madeira has, due to chance, two distinct parts: the first has to do with the field work, which describes what happened on site (description of the outbreak) and some of the implications of the event (including all the actions that have taken place to keep the quality and safety of blood components during and after the outbreak); the second part gives an interesting view of what is done on the day-to-day basis before a possible outbreak in pursuing that it does not occur (a perspective that elaborates on the various tests to verify the effectiveness of chemical, biological, biocides, insecticides, attractive or repellents products), and what should eventually be done before the first outbreak of any epidemic (the paper herein presented was obviously done a *posteriori*), it is not easy to anticipate future outbreaks but with a thorough analysis of the risks and current trends can frame the situations with the greatest potential and enclose them with all the knowledge already available but still dispersed.

See you soon!

Paulo Jorge **Nogueira, Diretor**

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Outbreak of dengue in Madeira:
context, epidemiological and entomological surveillance

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Abstract: This article describes the epidemiological pattern of dengue outbreak that occurred on the island of Madeira from September 26, 2012 to February 3, 2013. The epidemiological surveillance system is based on an active system of notification cases from week 44 of 2012 and in retrospective collection between weeks 39 and 43 of 2012.
The processing and analysis of data were intended to meet the trends of evolution of the outbreak concerning time, space, demographic characterization and symptoms of probable cases. From September 26, 2012 to February 3, 2013 a total of 2.165 probable cases were reported. During this period 1.080 cases were laboratory confirmed, corresponding to 50% of probable cases. From the "place of residence" was possible to identify the areas with highest incidence, at civil parish level. The cumulative incidence rate on the Madeira island was 80,85/10.000 inhabitants and the most affected municipality was Funchal with an incidence of 156,04/10.000 inhabitants.
The average age of probable cases was 39 years, with a standard deviation of 20 years. The most affected age group was that of 15-19 years, with an incidence of 134,26/10.000 inhabitants. The incidence in females was higher than in males with a value of 90,17/10.000 inhabitants. 127 cases were hospitalized, accounting for 5,9% of the probable cases. There was no report of severe cases or deaths. The epidemiological surveillance system remains on course and allowed the identification, per week, of suspected cases of dengue fever and the adoption of social mobilization measures, the *Aedes aegypti* vector control, with delimitation of areas with highest risk.

Keywords: **Dengue, Outbreak, Epidemiological Surveillance, *Aedes aegypti***

1 Introduction

Dengue fever is a viral disease, transmitted by mosquitoes, with the quickest spread rate in the world, and that leads the Arboviruses in humans [1-2] being considered an emerging disease with a geographic distribution, incidence and severity increasing in all tropical and subtropical regions in the world. It affects about 40% of the world's population[3].

Dengue virus (DENV) is diffused through a human-mosquito transmission cycle. Typically four days after being bitten by an *Aedes aegypti* infected mosquito, a person develops viraemia, a condition in which there is a high level of virus circulating in the bloodstream. The viraemia stands approximately for 5 days. Normally, symptoms arise 5 days after the bite and can endure for a week[4]. After the female mosquito feed from someone's infected blood, the virus multiplies over a period of eight to twelve days in the body of the arthropod.

From then on, the mosquito becomes a competent vector for transmitting the disease until the end of his life, which is six to eight weeks for the *Aedes aegypti*. The results of some studies

suggest that the dengue fever virus can be transmitted vertically, however these same studies point to the need of more research to further understand the transmission mechanisms [5].

The mosquito *Aedes aegypti* lives in urban areas and reproduces mainly in artificial breeding sites. It bites, particularly during the day and its activity peaks are early in the morning and in the hours before nightfall. The female bites several people during each feeding period. Both male and female mosquitoes feed on plant nectar, fruit juices and other plants sugars as their main source of energy. However, the female mosquito needs blood to produce eggs, which is why they bite humans. Each female mosquito can lay several batches of eggs during their lifetime and often take several blood meals before putting a lot of eggs. When a female mosquito is infected with the dengue virus, the virus is present in their salivary glands. The transmission of the virus from the salivary glands of the mosquito to a human being occurs when the mosquito initiates a blood meal, in which an infected female injects its saliva into the human host to prevent the host's blood to clot and to facilitate feeding [6].

In rare occasions the DENV transmission can be caused by blood transfusion or transplantation. The transmission never occurs between people. Most infections by the dengue virus are asymptomatic or may present a mild febrile framework. Most symptomatic cases resemble to flu. Severe and potentially fatal forms may represent 5% of the cases [7].

Symptoms arise on average 7 days after the bite (3 to 14 days) and include acute fever, intense headache, retro orbital pain, arthralgia, myalgia and exanthema. The symptoms typically persist for a 7 days period.

The DENV is constituted by viruses included in 4 distinct serotypes, forming a complex antigen. All viral serotypes may cause the same clinical frames [8].
The spread of dengue is attributed to the geographic expansion of the mosquito, responsible for causing the disease, associated to their increased interaction with humans and their urban behaviour. More than half of the world's population lives in countries endemic for dengue. There is currently no available vaccine for dengue.

1.1 Geography

Located in the Atlantic Ocean between parallels 30° 01‘ N and 32° 24‘ N and the meridians 15° 51‘ W and 17° 15‘ W, the Madeira Archipelago comprises the Madeira and Porto Santo islands and the nature reserves Desertas Islands (Ilhéu Chão, Deserta Grande and Bugio) and Selvagens Islands (Selvagem Grande and Selvagem Pequena). This archipelago is, along with the Atlantic archipelagos of Azores, Canary Islands and Cape Verde the biogeographically region of Macaronesia. Madeira Island has an area of 756.5 Km^2. Measures in its greatest length 57 Km, between Ponta de São Lourenço and Ponta do Pargo (East-West), and has a maximum width of 23 kilometers in the direction North-South, between Ponta de São Jorge and Ponta da Cruz. It is approximately 845 Km from Portugal Mainland and about 650 kilometers off the North coast of Africa. The island is administratively divided into 10 municipalities and 53 civil parishes, being the capital city Funchal. The municipality of Funchal is located in the southern half Madeira island and has as limits: to the North, the peaks of the central mountain massif that divides the north and south coasts of the island; to South, the Atlantic Ocean; to West, Ribeira dos Socorridos and to East, Ribeira da Quinta, which separates the civil parish of São Gonçalo from Caniço. Administratively it is divided by 10 civil parishes. The morphology of the scenery in which the Funchal urban area deploys, presents itself as a “large amphitheatre”, which rises steeply from sea level up to a hilly set that culminated in addition to 1800 meters above sea level, in Pico do Areeiro (1818 m) [9].

1.2 Climate

The climate in Madeira is strongly conditioned by the intensity and location of the Azores' anticyclone, being the orography, configuration and orientation of island preponderant factors in the genesis of the island different microclimates that exist throughout the island. The rugged relief, beyond the effect of the altitude, induces local climate sensitivity, due to the elongated configuration of the island and its East-West orientation, perpendicular to the direction of the prevailing wind from the North. These constraints generate air temperatures and precipitations distinct to the same height on strands with different exposure to prevailing winds. The region has a mild climate based on an average annual temperature 19.0° C and a thermal amplitude of just 6.5 ° C. August is the hottest month (22.6° C) in counterpoint with February, which presents itself as the coolest month (16.1° C). With altitude to grow are microclimates, which promote a notable variation of vegetation. The annual averages of precipitation increases with altitude, being, as a rule, higher in the northern slope compared to registered on the southern slope,

to the same altitude. The highest average annual precipitation values are recorded in the meteorological stations of Bica da Cana (1560 mm) and Areeiro (1510 mm) with maximum totals close to 3000 mm/year; the annual average rainfall for the whole Madeira island is 1636 mm. Compared to the values registered over the ocean, the averages of the nebulosity on Madeira are higher, due to the formation of orographic clouds and fogs. The maritime humid air "collides" against the island (mountainous barrier perpendicular to the prevailing wind direction) and is forced to climb in altitude, adiabatically cooling and condensing into small particles suspended in the atmosphere, which causes clouds or fog, depending on the condensation play in altitude or along the surface [9].

1.3 Demographics

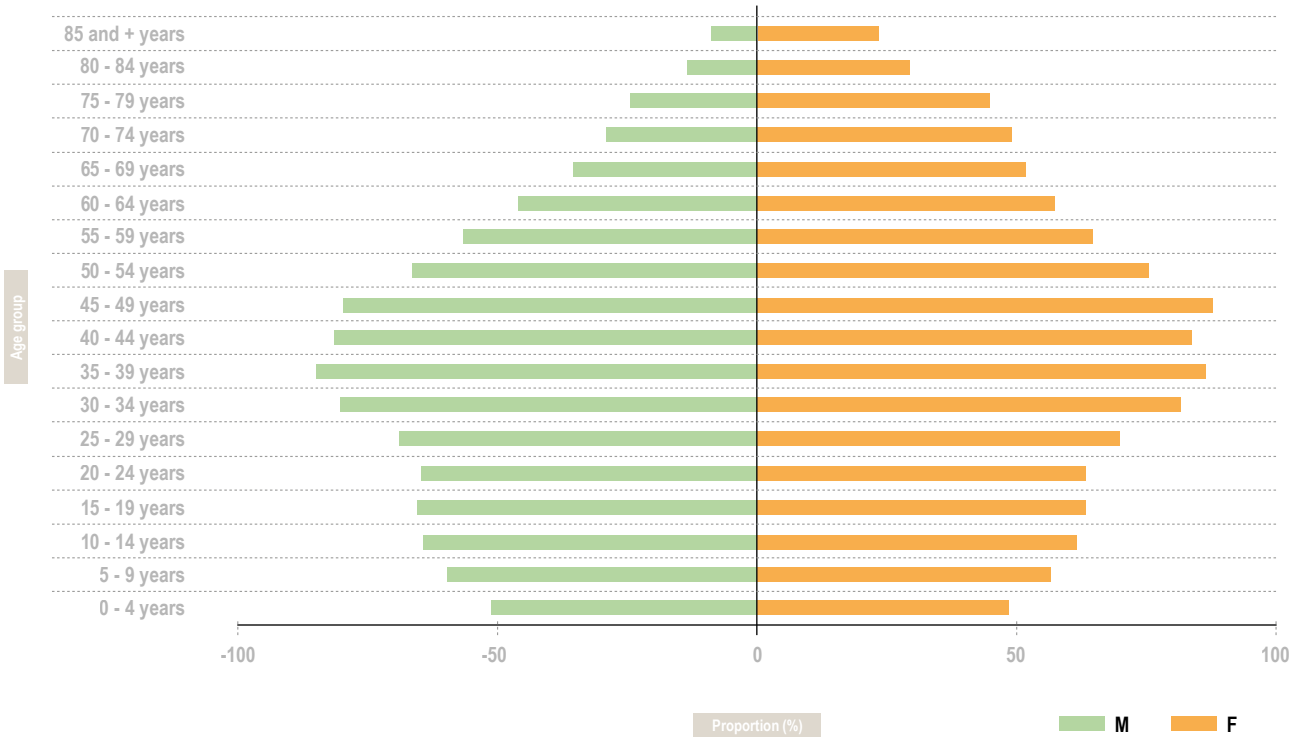
According to the Census of 2011 the Autonomous Region of Madeira (RAM) has a resident population of 267.785 inhabitants, where about 47% of individuals are male and 53% are female. Concerning the distribution by age group, both male and female individuals have greater representation in age groups between 30 and 49 years *(figure 1)*.

The population density of the Madeira Archipelago is 334,3 inhabitants/km2 and in the Funchal municipality is 1.469,6 inhabitants/km2, corresponding to the municipality with the RAM's highest population density[10]. It should be noted that the area used to calculate the Madeira Archipelago population density was its very area, whose value is 801 km2.

The RAM regional health service consists of 7 hospitals, where 1 is public and 6 are private and the number of Health Centres is 49.

Figure:

① Age pyramid of the population resident, RAM, 2011



1.4 Sanitary Conditions

The increase of urbanization, as registered all over the world, mostly with urbanization processes few ruled and deficient from the point of view of health, seems to contribute to the spread of dengue, which even without registering fatal ways, brings significant economic and social costs like absenteeism, immobilization, debilitation and medication [11].

It is important to mention that Madeira has no urbanization or sanitation problems in most of the urban areas. Concerning hygiene conditions, indicators obtained on Madeira point to 99% of the resident population served by water supply system (public and private) and 96% of the resident population served by wastewater drainage systems [10].

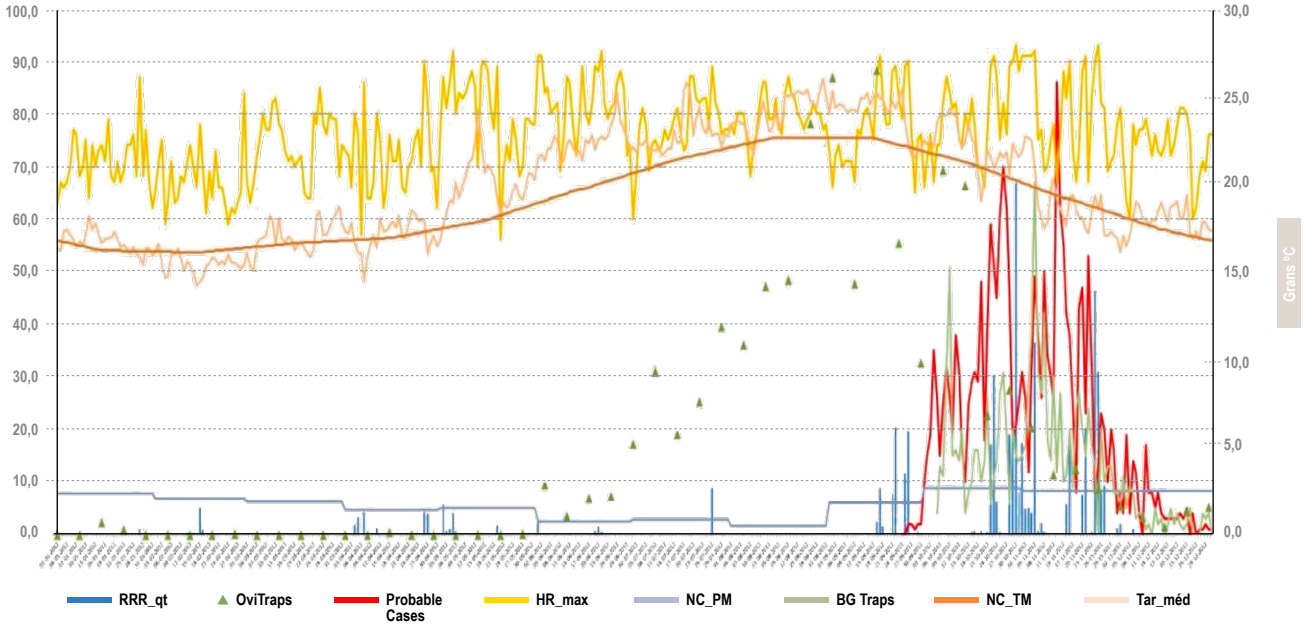
1.5 Entomological Findings

Since October 2005 the population of the mosquito *Aedes aegypti* Linnaeus (Stegomyia), 1762 has been expanding in the municipality of Funchal [12]. In 2008, the mosquito was detected in the municipalities of Câmara de Lobos and Santa Cruz [13].
In September 2011, an entomological crossed study was held using a total of 273 traps: Madeira (253 ovitraps), and Porto Santo (20 ovitraps). The results demonstrated then that the mosquito population expanded westward (municipalities of Ponta do Sol and Ribeira Brava) and maintained its presence at East of the city of Funchal, i.e., in the municipality of Santa Cruz. In this study, the presence of the vector *Aedes aegypti* was also verified in Porto Moniz, but subsequent monitoring activities did not sensed its presence in that municipality [14].

In most countries with history of outbreaks, they occur in the rain season coinciding with the hot and wet season. From data collected of the network of oviposition traps and pitfalls of adults capture, who constitute *Aedes aegypti* vector surveillance network and monitoring of the air temperature, humidity and rainfall values, was noted that in the period preceding the appearance of dengue first cases, an increase of rainfall was recorded and consequently an increase in the number of eggs and adult midges *(figure 2)*. Practically non-existent rainfall in more than 2/3 of the calendar year in study recorded several days of rain during the outbreak period. Diseases caused by vectors like dengue fever are particularly sensitive to climate fluctuations and changes, at global and local level, because the vector biology and viral replication are dependent of humidity and temperature [15].

Figure:

② Daily rainfall, daily average temperature, maximum daily relative air humidity, climatological normals (1971-2000), number of female adult mosquitoes and of *aedes aegypi* eggs, probable cases of dengue fever * 3 (2012)



RRR -Total daily rainfall (mm) **Ovitraps** - Oviposition traps **HR** - Relative humidity **NC_PM** - Climatological normal indicating the average amount of total precipitation (mm).
BG Traps - Traps for female adult mosquitoes **NC-TM** - Climatological normal indicating the average air temperature (° C) **Tar** - Average air temperature

Source of climatological data: Meteorological Observatory of Funchal

2

Material and Methods

The implemented epidemiological surveillance system was based on data collected retrospectively between weeks 39/2012 and 43/2012, on the clinical processes records of RAM Health Service, Public Enterprise Entity (SESARAM, E.P.E.), on the on laboratory services available data also from SESARAM, E.P.E, related to blood samples, indicating the confirmed cases. From week 44/2012 onwards, the epidemiological surveillance system was based on an online survey filled at the time of clinical approach, with all required fields, including fields for collecting sociodemographic information like place of residence or place of daily activity. The survey collects information on clinical and epidemiological component admitting, or not, that the case meets the clinical and epidemiological criteria of definition of probable case, according to the normative circular 6/2012 of 11/9/2012 of the Institute of Directors of Health and Social Affairs, IP-RAM [16].

In sequence the following definitions were taken.

Probable case is defined by the presence of clinical and epidemiological criteria, where clinical criterion corresponds to patient with acute fever and at least two symptoms among headache, retro orbital pain, myalgia, arthralgia, exanthema, haemorrhagic manifestations or leukopenia.

The epidemiological criteria correspond to reside or remain in a region affected in the 21 days prior to the occurrence of symptoms.

The system also monitors the confirmed cases, defined as any probable case with one of the following techniques of laboratory confirmation:

- Serological Response (IgM): specific to dengue virus in blood or cerebrospinal fluid (CSF), neurological manifestation occurred;
- IgG seroconversion to dengue virus;;
- Isolation or nucleic acid detection (RT-PCR) of dengue virus in the bloodstream or LCR

The implemented epidemiological surveillance system still considers imported cases that are based on the following definition: case that meets clinical criteria, laboratory evidence of recent infection by dengue virus and travel history to endemic zone or with active outbreak of dengue fever in the last 21 days [17].

The data collected through the implemented system were subjected to a descriptive statistical analysis. Epidemiological measurement, incidence rate per 10.000 inhabitants, was calculated according to the municipality of residence, age group and sex, using the results of the 2011 Census regarding the population resident in RAM. Data processed and analysed gave rise to the several bulletins that were broadcast together from regional and national health authorities, namely the Directorate-General of Health (DGS) which referred them to international health organisations.

Climatological, entomological and epidemiological available data were analysed and combined in order to contribute to the construction of a model of risk prediction of outbreak eclosion. The combination of data is represented in the graph 2 (integrated in point 1.5. Entomological Findings). Daily precipitation variables, daily average temperature, maximum daily relative air humidity, climatological normals 1971-2000 (daily average temperature and daily average amount of precipitation), number of female adult mosquitoes and number of *Aedes aegypti* eggs and probable cases were considered. Note that only the probable cases are multiplied by 3 for a graphical representation of the scale itself. Regarding ovitraps (traps for *Aedes aegypti* eggs) the weekly average number of eggs collected in 55 of the 81 oviposition traps distributed by the Funchal municipality is represented. Calculation is obtained based on the median of the total number of traps read during the referred week dividing the total number of eggs by this value.

3

Results

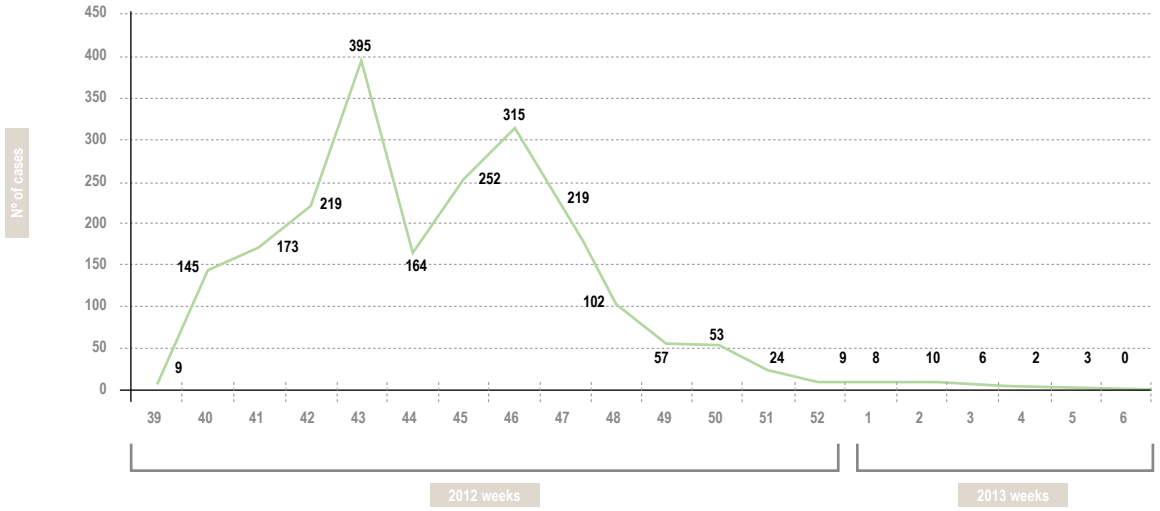
All data was analysed in order to evaluate development trends of the outbreak in terms of time, space, sociodemographic characterisation and symptomatic presentation of probable cases.

In the period under review, from September 26, 2012 to February 3, 2013, 2.165 probable cases were reported. The first two confirmed cases of dengue fever occurred in September 26, 2012, more precisely in week 39/2012. The epidemic curve *(figure 3)* shows the increasing trend in the number of probable cases of dengue fever between week 39/2012 and 42/2012. The rise in the number of probable cases occurred in week 43/2012, in which 395 cases were notified. Despite the decrease recorded in week 44/2012, the data related to the following two weeks, weeks 45/2012 and 46/2012, show an increase in the number of probable cases when compared with the first 4 initial weeks of the outbreak. From week 47/2012 there is a decreasing trend of the

outbreak, registering a progressively decrease in the number of probable cases until week 6/2013 with 0 case. In general, most of the observed probable cases occurred between weeks 42/2012 and 47/2012. From the three reported cases in week 5/2013, only one has been laboratory confirmed and was identified as an imported case from Brazil.

Figure:

③ Number of dengue fever probable cases from week 39/2012 until week 6/2013, in RAM



The cumulative incidence rate on the Madeira island was 80,85/10.000 inhabitants and the Funchal municipality was the most affected with an incidence of 156,04/10.000 inhabitants *(Figure 4)*.

The average age of probable cases is 39 years and the standard deviation is 20 years compared to the average age, which means that the majority of patients are aged between 19 and 59 years.

The most affected age group was that of 15-19 years, with an incidence rate of 134,26/10.000 and the least affected were those from 0 to 4 years of age (35,74/10.000 inhabitants) *(Figure 5)*.

Figure:

④ Distribution of cumulative incidence rate of dengue fever probable cases from week 39/2012 until week 05/2013 inclusive, per parish, in RAM

Legend **Cumulative Incidence /10000 hab**

No cases | 0-10 | 10-50 | 50-100 | 100-200 | 200-500 | > 500

Figure:

⑤ Cumulative incidence rate of dengue fever probable cases by age group, from week 39/2012 until 5/2013, in RAM

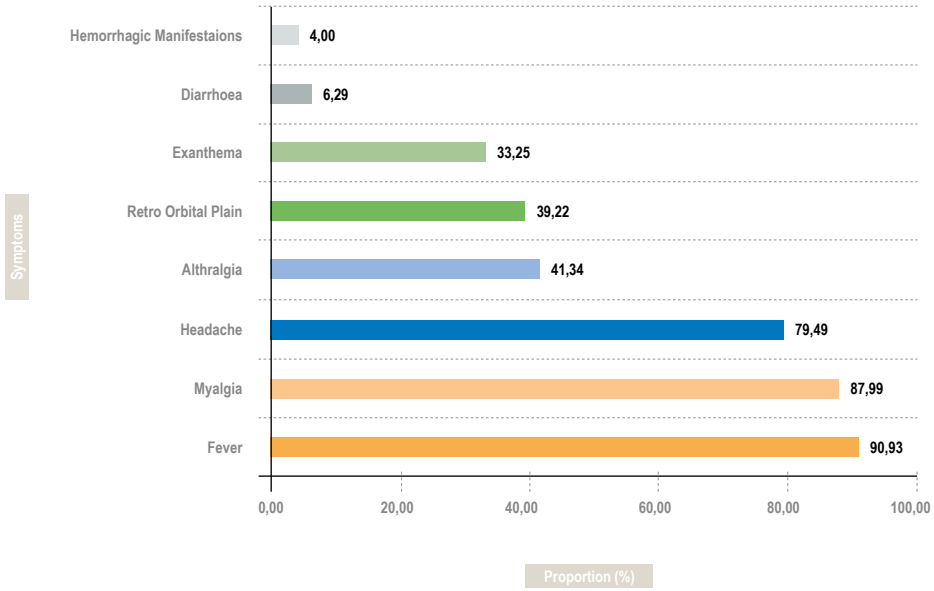
The male/female ratio was 0,70, while the female represented 59% of all persons affected. The incidence rate in women was 90,17/10.000 inhabitants and in men was 70,41/10.000 inhabitants.

During this period, a total of 127 cases were hospitalized corresponding to a hospitalization rate of 5,9%, compared to the probable cases.

By information on the obtained symptomatology for all probable cases and as these data were collected only from week 44/2012 onwards, we found that most frequent symptoms were fever, myalgia and headache. Diarrhoea and haemorrhagic manifestations are the ones who have less reference, however is pointed out that 6,29% of registered cases from week 44/2012 onwards presented diarrhoea *(figure 6)*.

Figure:

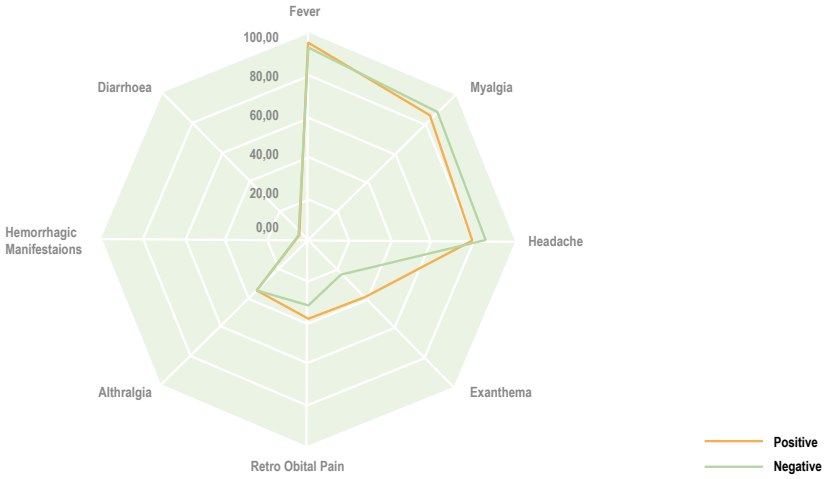
6 Frequency of symptoms presented in probable cases, between week 44/2012 and 5/2013, in RAM



On the other hand, the symptoms recorded among laboratory confirmed cases and negative cases are similar, and this similarity is understood by the fact that the recorded light to moderate frames, showed a discrete symptom picture according to the description of other outbreaks in literature *(figure 7)*.

Figure:

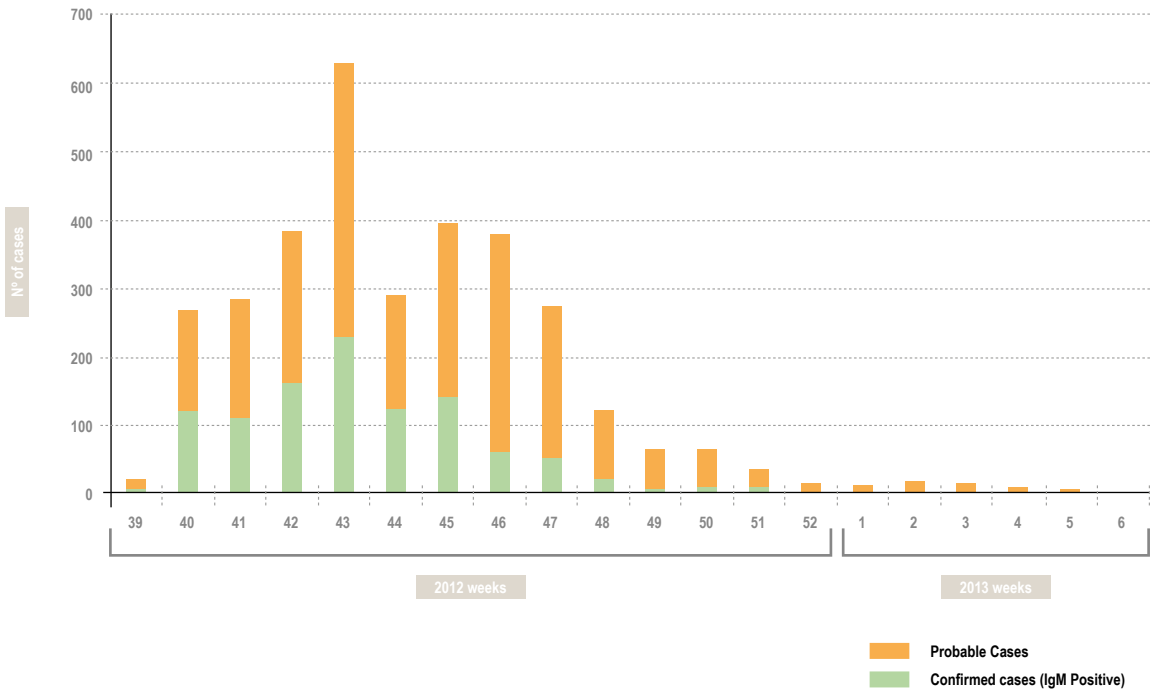
⑦ Symptoms frequency in positive and negative cases, between week 44/2012 and 5/2013, in RAM



In the period of the outbreak 1.080 cases were confirmed in the laboratory, which corresponds to 50% of the probable.

Figure:

⑧ Probable dengue fever cases and laboratory confirmed (igm), between weeks 39/2012 and 6/2013, in RAM



From all the cases investigated in laboratory, 43% were confirmed cases, 17% were negative and 0,8% were doubtful. Concerning cases that were not analysed, as defined in methodology, 1,1% were cancelled, 0,2% were based on pending cases, 30,7% were dengue zone rejected and 7% were rejected.

4

Conclusion

The regional context in terms of the *Aedes aegypti* vector presence, sociodemographics and climate conditions, puts the RAM at risk due to the emergence of an arbovirose transmitted by the *Aedes aegypti* vector. The incidence and outbreaks of dengue fever in the world have been associated with the rainy seasons. Climatological data, empirically, seem to corroborate this association also in RAM.

The incidence of dengue cases was greater in the urban environment and affected more women than men, hitting more population aged between 15 and 19 years. No severe cases or deaths were recorded and hospitalized cases fit in a secondary prevention measure for better follow-up of cases whose individual condition pointed to greater potential for less favourable evolution.

Since the onset of the outbreak, Health Services in RAM built from the extensive knowledge of the dengue vector transmitter in Madeira, the systematization of statistical data collection and processing and dissemination of processed information, a timely response to the outbreak with support of internal and external partners [18].

Timely knowledge of the location of areas with higher incidence allowed to guide and adopt control measures focused to reduce places favourable to the proliferation of the mosquito population. This first outbreak of sustained dengue in a European Union region invokes the need of the development of integrated response strategies to mosquito-borne diseases invaders and a system implementation of the same regions at highest risk.

Conflict of Interest

The authors declare no conflict of interest.

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5

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English Version B

Dengue Outbreak in Madeira Island.
Blood Quality and Safety Measures.

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Abstract:

Introduction - On 3 October 2012 two cases of confirmed dengue infection were reported by Autonomous Region of Madeira in patients residing in Madeira Island, this was the onset of the first recorded Dengue outbreak in this island. Most Dengue infections are asymptomatic. Dengue has been identified as a potentially serious threat to the blood quality, safety and supply and has been the focus of many recent studies trying to evaluate its transfusion transmission risk, as well as the prevalence of asymptomatic viremic blood donors.

Material and Methods - A crisis management team within the Portuguese Blood and Transplantation Institute, the Competent Authority for blood and transplantation - *Directorate General of Health*, *National Health Institute Dr Ricardo Jorge* and *Hygiene and Tropical Medicine Institute* experts, issued recommendations on precautionary blood quality and safety measures.

Results and Conclusion - During the study period, there were no reports of post transfusional Dengue infection to the Portuguese Haemovigilance System. Data demonstrate that viremic blood donors, in the acute phase of infection, can remain asymptomatic and present as healthy donors.The rates of dengue viremic donors reported in this issue are higher than those in previous reports (Puerto Rico, Brazil). Although the impact of the blood quality and safety measures on the blood supply was moderate a surveillance and alert system is required.

Keywords: **epidemiological surveillance, outbreaks, Dengue Fever, blood safety, patient safety.**

1 Introduction

On 3rd October 2012 two laboratorial confirmed cases of dengue fever were reported by Autonomous Region of Madeira in patients residing in Madeira Island. Both patients had no recent travel history abroad. Although the presence of aedes aegypti in the Island was known since 2005 this was the first known occurrence of autochthonous Dengue fever.

By 3 March 2013 the outbreak was considered under control, 2187 cases had been reported in Madeira Island. Another cases had been reported, 11 cases in Portugal mainland and 71 in thirteen European countries, all among travelers returning from Madeira. There were no registered deaths. The case number and incidence rate has decreased to residual values since the beginning of 2013 and there were no laboratory-confirmed dengue case since the 4th February. (1)

Dengue is among the most important mosquito-borne viral diseases in the world and its incidence has increased in the last 25 years. Most Dengue infections are asymptomatic (53%-87%(2)

Identified as a potentially serious threat to the blood quality, safety and supply, Dengue has been the focus of many recent studies trying to evaluate its transfusion transmission risk as well as the prevalence of asymptomatic viremic blood donors, in the acute phase of infection.

Previous studies have identified three well-documented transfusion transmitted Dengue cases in Hong Kong (2002), Singapore and Puerto Rico (2007) as well as moderate rates of dengue viremic asymptomatic blood donors in Brazil, Honduras and Puerto Rico. (2-7)

The aim of this study was to describe the precautionary health measures adopted for blood quality and safety, to assess the existence of post transfusional Dengue reports and the prevalence of asymptomatic dengue infection among healthy blood donors.

2

Material and Methods

A crisis management team within the Portuguese Blood and Transplantation Institute, represent by the Haemovigilance Steering Committee, the Competent Authority for blood and transplantation - *Directorate General of Health, National Health Institute Dr Ricardo Jorge* and *Hygiene and Tropical Medicine Institute* experts, issued the following recommendations on precautionary blood quality and safety measures:

Nationwide measures for protecting the blood system included: dissemination of Dengue information to all Blood establishments, in Portugal mainland deferral of potential blood donors who visited Madeira island for a period of 28 days after leaving the affected area, deferral of potential blood donors with fever or flu-like symptoms for 28 days after recovery, deferral of confirmed cases of dengue infection for 120 days after recovery, enhancement of post donation information and post transfusion haemovigilance[4]

Local measures applying to the affected area blood establishments included: quarantine of all red cell concentrates resulting from blood collected during the previous 28 days and retrospective testing, reverse transcription – polymerase chain reaction (RT-PCR) laboratory screening test for all blood donations after 4th October, information to blood donors to report any symptoms in the 15 days after donation, stop of platelets production, platelet pools were supplied by Portuguese Blood and Transplantation Institute.

It was recommended a special attention to the optimal use of blood components and appropriate management of the blood supply.

The Portuguese recommendations on measures for blood safety were more restrictive than European Centre for Disease Control (ECDC) Rapid Risk Assessment recommendations [8], which referred only to preventive measures at the local level: deferral of Dengue confirmed cases and potential blood donors with fever or flu like symptoms for a period of 28 days after recovery, quarantine of blood components on stock for 72 hours and possibly pathogen reduction in platelets and fresh frozen plasma. Additional blood donation screening for asymptomatic infection of the donor was also considered.

However on 21 November against the existence of 32 reported cases in Portugal mainland residents and European tourists, the ECDC has recommended the European countries Competent Authorities the deferral of potential blood donors who have visited Madeira Island for a period of 28 days.

3

Results

Precautionary Nationwide Measures

Performing their activity in Portugal 30 Blood Establishments, it couldn't be assessed, during the outbreak period, the number and incidence of potential blood donors deferred for having visited Madeira Island for 28 days after they have left the affected area, with fever or flu like symptoms. There were no reports of post transfusional Dengue to the Portuguese Haemovigilance System during the study period.

Precautionary Local Measures

The retrospective donor serological screening of the quarantine components detected 9 IgM positive cases (20,9/1000 donations) and 7 IgG positive cases in 439 donors.The first IgM positive identified cases were related to three donors who gave blood on 11 September 2012, 23 days before the outbreak onset. .

RT–PCR screening of 1948 blood donations during the period 06/10/2012 - 13/02/2013 detected 44 Dengue (DEN) positive cases, in asymptomatic donors who present to blood donation with a rate of 22,6/1000 donations.*(Table1)*

Table:

① Distribution and incidence of DEN RT-PCR positive cases 2012- 2013

Período	Screening Institution	Blood Donations (n)	RT-PCR DEN positive cases	Incidence rate
06-10-12 a 05-11-12	INSA	397	6	15/1000
06-11-12 a 30-11-12	Hospital Dr Nélio Mendonça	392	15	38/1000
01-12-12 a 31-12-12	Hospital Dr Nélio Mendonça	437	21	48/1000
01-01-13 a 31-01-13	Hospital Dr Nélio Mendonça	531	2	3,7/1000
01-02-13 a 13-02-13	Hospital Dr Nélio Mendonça	191	0	–
Total		1948	44	22,6/1000

It was identified the presence of DEN-1 vírus by National Health Institute Dr Ricardo Jorge. Sequence analysis of viral genome indicates high sequence similarity with viruses circulating in Latin America.

Since 31 January 2013 there were no positive DEN cases in blood donors.The number of cases in the population also began to decrease to residual values since the beginning of 2013, does not have been reported, since February 4, any laboratory confirmed case.

4

Conclusions

The Portuguese recommendations on measures for blood quality and safety were more restrictive than ECDC Rapid Risk Assessment recommendations. Given the results of the retrospective serological screening these recommendations seem to have been appropriate.

Data demonstrate that viremic donors, in the acute phase of infection, can remain asymptomatic and present as potentially healthy donors.

The rates of dengue viremic asymptomatic donors reported in Madeira Outbreak (2,26%) is much higher than the reported in previous studies but similar to the incidence rate in the population (Funchal).

Prevalence studies in asymptomatic blood donors screened by RT- PCR in endemic areas, found prevalence rates of 0,06% in Brazil [4] ,0,07% in Puerto Rico [4] and 0,4% in Honduras. [3] The highest prevalence rates reported in medical literature refer to the occurred during Dengue outbreaks in Puerto Rico (2007 and 2010) and Ribeirão Preto, Brazil, (2010), in which the rates of viremic donors ranged between 0,19% 0,45% and 0.4%, respectively [2].

The combination of the high vector density with multiple breeding sites in the city and the lack of the immunity of the population are likely to explain the dimension of the outbreak.

Although the impact of the blood safety measures on the blood supply was moderate, preparedness for the coming years is essential. The epidemiology in Europe might be changing, more than ever epidemiological and laboratorial surveillance systems are required.

Conflict of Interest

The authors have no conflict of interest to declare.

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To National Health Institute Dr Ricardo Jorge collaborators: Dra Maria João Alves, Dra Sofia Núncio

To Hygiene and Tropical Medicine Institute collaborators: Dr Paulo Almeida, Dra Carla Sousa.

5

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p e r s p e c t i v e s

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Biocides: Issues surrounding the use of insecticides and repellents in the control of vector borne diseases

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Summary: The effectiveness of biocidal products against disease vectors is crucial to reduce the morbidity and mortality observed as a result of vector-borne diseases. This article discusses key aspects relating to the testing of biocide products in order to fulfil European regulatory requirements to facilitate their use in disease vector control.

Keywords: **Dengue; insecticides; repellents; laboratory tests; field trials; effectiveness**

1 Introduction

Dengue infection is caused by a flavivirus. The virus is transmitted to susceptible individuals through the bite of mosquitoes of the genus *Aedes*, and in particular *Aedes aegypti* infected with the virus. The control of Dengue necessitates a comprehensive series of public health measures including; environmental monitoring, habitat control, education and strategies to limit the importation of vectors. A substantial reduction in the transmission of Dengue can be achieved by;

- The use of insecticides to reducing the population of *Aedes aegypti;*
- The use of repellents to reduce the number of mosquito bites.

A biocide is a chemical or biological substance which can deter or control a harmful organism. This article is concerned with chemical and biological biocides including insecticides, insect attractants and insect repellents.

All biocidal products to be placed on the market in the EU require authorization. Authorization occurs at a member state level and can only be obtained once the active substance contained within the biocidal product has been approved under the EU Biocides Regulation 528 / 2012 (EU BPR)[1]. One of the aims of the EU BPR is to harmonize information requirements for the approval of an active substance across the European Union. Study data and other product information must fulfil the minimum requirements outlined by the EU BPR in order that a risk assessment can be conducted before the approval of an active substance or authorization of a biocidal product. Authorized biocidal products must also comply with the Classification, Labelling and Packaging (CLP) regulation 1272/2008[2] which aims to harmonize the communication of hazard information about chemicals.

Adhering to these regulations help manufacturers and formulators demonstrate, at a given concentration, the effectivity of a biocidal product, the consistency of effectiveness and cost effectiveness. These regulations also serve as an assurance of the risk and risk mitigation measures that are specified on the product label.

To ensure that only effective products enter the market, manufacturers seeking approval must submit data which demonstrates the efficacy of the active agent against specified target organisms at the dose for which approval is being sought. These data form a key component in the decision making process for approving active ingredients or authorizing biocidal products. Products are only approved or authorized if they demonstrate a sufficient level of proof of efficacy.

The objective of this paper is to describe the range of effectiveness studies. As biocides are intended to control living organisms, they can pose risk to human or animal health and have significant and long lasting adverse effects on the environment. We define effectiveness as the ability of the proposed biocidal product to produce its intended effect measured against the risks that the proposed product poses to humans, animals and the environment[3].

2

Patterns of tests available

An evaluation of the effectiveness of a biocide product is distinct from the evaluation of efficacy of the active substances contained within it. Whilst tests efficacy examine the intrinsic effects of the active substance, an evaluation of the effectiveness of a biocidal product takes a broader perspective. It necessitates consideration of all the components of the product, not just the active substance. It takes into account a number of features of the product; the organism to be targeted, the effect on the organism (kill, control, repel or attract), location in which the product can be used (indoor, outdoor or both), directions for product use, risk mitigation measures defined for the product, the time interval to be observed between applications and the shelf live and storage recommendations for the product.

When submitting a dossier of evidence for the authorization of a biocidal product, the product label should be included as part of the data set submitted, in addition to the protocols used and evidence from laboratory tests or field trials. The label of a biocidal product cannot make claims about a specific disease, only the vector involved.

The tests with mosquitoes should normally be made with Culex spp or *Aedes* spp as these are the most aggressive. If the product is aimed controlling a disease vector, then it is necessary to test the efficacy of the product against the specific species of mosquito for example, if the product is designed to control the vector responsible for dengue and yellow fever then the product must be tested for efficacy against *Aedes aegypti* and *Aedes* albopictus.

3

Requirements for testing and standards of effectiveness by product type

Insect repellents are products whose mode of action is to deter and repel mosquitoes. The purpose of laboratory studies of the repellents is to estimate the dose and time of protection provided by an application of the repellent. These products can be applied to human or animal skin, applied to clothing or released to the environment. Studies of repellents in humans are the method of choice1 because they most closely approximate the intended conditions in which the product will be used.

Insecticides are products which kill or control insects, including mosquitoes. These products can be applied to surfaces or released into the environment.

Insect traps such as CDC miniature light trap are widely used as a survey tool for mosquito control operations. These traps provide quantitative data (number of mosquitoes captured) and qualitative (which species present in the catch). Other capture methods, including those using aspirators can be used.

During field and simulation tests, use of the biocidal product should respect the specified instructions for application, particularly in terms of the recommended concentration. Not all biocidal products require field tests if the intended location of use can be successfully simulated in the laboratory. Climatic conditions can influence the effectiveness of some biocidal products, for example larvicides to be used in swamps and lakes or biocides intended for aerial release, field tests are strongly recommended in order to determine effectiveness.

The timing of field trials is crucial to determining effectiveness. Field studies for mosquito control should be conducted during the spring and early summer as during autumn the population of adult mosquitoes naturally decreases, therefore making it more difficult to attribute the decline in population to the use of the biocidal product. Field studies for larvicides should be conducted during July and August, when there are sufficient levels of *Aedes* Spp and *Culex* spp. The studies listed below[1] are not an exhaustive checklist or rigid criteria for obtaining decision on the effectiveness of a biocidal product. Expert advice should also be considered and used either in the design of trials or in the evaluation of results.

3.1 Standards for insecticides and repellents intended to be used on surfaces or for indoor vaporization:

- Laboratory tests should demonstrate mortality in adult mosquitoes after 24 hours > 90%;
- Simulation tests demonstrating inactivity and mortality and / or residual effectiveness (depending on the specific claims of the product) > 80%.

3.2 Standards for larvicides:

- Laboratory tests should demonstrate a larval mortality of 100% after 24 hours of contact. Slow-acting products which show a 100% mortality after 48 hours, 72 hours or even longer can be considered.
  In exceptional circumstances, products can be considered which demonstrated a larval mortality of <90% if all remaining larvae subsequently died before or during emergence.
- Simulation tests must reflect a decrease in the number of emerging mosquitoes by 80%;

- Field trials are obligatory if the intended area of use is the treatment of surface water. These field trials must demonstrate a larval mortality > 90% or a decrease in the number of emerging mosquitoes by 80%.

3.3 Standards for skin or clothing repellents[4]:

- Simulation tests demonstrating with the use of insect traps, the efficacy of the repellent;
- Field trials demonstrating the effectiveness of the repellent.

The effectiveness of the repellent is defined in terms of the time elapsed between application of the product to the second bite or their time from onset of exposure to the first confirmed bite. If this time is shorter than that stated by the product manufacturers, this information must be included in the risk mitigation measures of the product to avoid disease transmission.

3.4 Standards for repellents not intended for use on skin or clothing:

- Laboratory test and / or test simulation which demonstrate a repellent effect of > 80%
- Depending on the intended use, field trials should demonstrate a repellent effect of about 80%.

3.5 Standards for repellents intended for use on horses;

- Laboratory test which demonstrates the repellent effect;
- Simulation or field tests which demonstrating the repellent effect against mosquitoes of the specific target species in target animals over the period claimed. The repellent effect should be 90%, if not, data must be submitted to allow the calculation of the "full-time protection", defined as the time until the next bite.

The simulation tests may be waived if a robust field trial is submitted.

4 Final Thoughts

Biocidal products are an important means of controlling organisms which pose a threat to human health. It is important that there are safeguards to ensure that biocide products can be used without causing harm to people, animals or the environment. The development of specific study methods to assess the efficacy of the active substance, assess the safety of biocidal products and ultimately stipulate conditions for the use of biocidal products to ensure their continued availability to support public health interventions in the fight against vector borne diseases.

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English Version D

Was it possible to anticipate the intensity of the Madeira's dengue epidemic for the year 2013?

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Abstract: The dengue virus has had a detrimental effect on the inhabitants of many tropical areas, infecting millions of people worldwide. The dengue epidemic in the Autonomous Region of Madeira, a Portuguese archipelago, was officially declared by the Portuguese Health Authorities on the 3rd October, 2012.
The main objectives of this study were: **1)** to better understand the evolution of the dengue fever in Madeira and **2)** to anticipate possible scenarios for the year of 2013. This study was more challenging due to the absence of past evidence because it referes to the first epidemic of this kind on the island. Therefore, past information regarding outbreaks of this epidemic in other parts of the world was gathered to trace the behavior of the virus itself. Then, six regions of the world were selected according to predefined criteria that reflected similar ambient conditions to those of Madeira during the spread of the epidemic. It was concluded that in 2013, between **2160 and 2470 dengue clinical cases** were expected to occur in the archipelago. Up until the 3rd of February of 2013, a total of 2164 clinical cases were in Madeira.

Keywords: **dengue epidemic, dengue virus, attack rate, mortality rate, population density, subtropical climate**

1 Introduction

Dengue is a disease caused by a virus, DENV, and it is transmitted to a person through bites from the *Aedes Aegypti* mosquito, which generally breeds in big water containers. However, dengue can also be transmitted by the species *Aedes albopictus*, which breeds in small containers, such as flowerpots. The big urban centers have been the most convenient places for these mosquitoes to find their principal source of food: human blood. The disease is prevailing in the southeast regions of Asia and recently has become more and more common in the tropical countries of the Pacific, Oriental Africa and Latin America[1]. As dengue viruses infect millions of people each year, this disease is considered to be one of the most important infectious diseases in many tropical and subtropical countries. In fact, research indicates that about 40% of the world's population lives in countries at risk of dengue infection[1].

Although cyclic, the incidence of this disease is increasing and spreading geographically[2]. There can be many causes for such a trend: for instance, an increase of the reported data, improved diagnostic tools, along with higher levels of urbanization. The tendency of countries becoming increasingly urbanized is clear, and with mosquitoes thriving in large cities, this could lead to a greater concentration of insects and hence a growth in dengue incidence. On the other hand, technologies, allowing a more efficient report of data, improved over time. Obviously, with more data reported, there will be better knowledge of dengue cases occurrence.

The dengue virus' incubation period is about 3-7 days, but it can last up to two weeks [1]. The virus can cause different symptoms on the humans. Usually it is flu-like, but in severe cases it can lead to death.

Theoretically, the number of people infected by the virus is expected to increase during periods characterized by extreme heat, substantial precipitation and relative humidity. These are the perfect conditions for the mosquito (host) to reproduce. Still, it should be taken into account that the rainfall cannot be too high, otherwise it may flood the breeding niches.

The Autonomous Region of Madeira is a Portuguese archipelago, with 267 785 inhabitants (2011)[3], located 520 km from the African Coast and 1,000 km from the Continental Europe. Madeira has an *infant mortality* rate of 3.4 per thousand[4], a *population density* of 300/km^2[5] and an *urban population* of about 70%. This last value is related to the most populated cities of Madeira: Funchal,

Santa Cruz and Câmara de Lobos (contributing to a total of 0.19 million inhabitants[6]). The archipelago's subtropical climate is influenced by its geographical position, although there are clear weather variations between the northern and the southern regions. Madeira has a microclimate with average temperatures rarely falling outside the range of 13°C – 25°C. The hottest months are August and September, while the four month period between November and February has the highest average of rainfall (*Figures 1*[7], [8] and *2*[7], [8]).

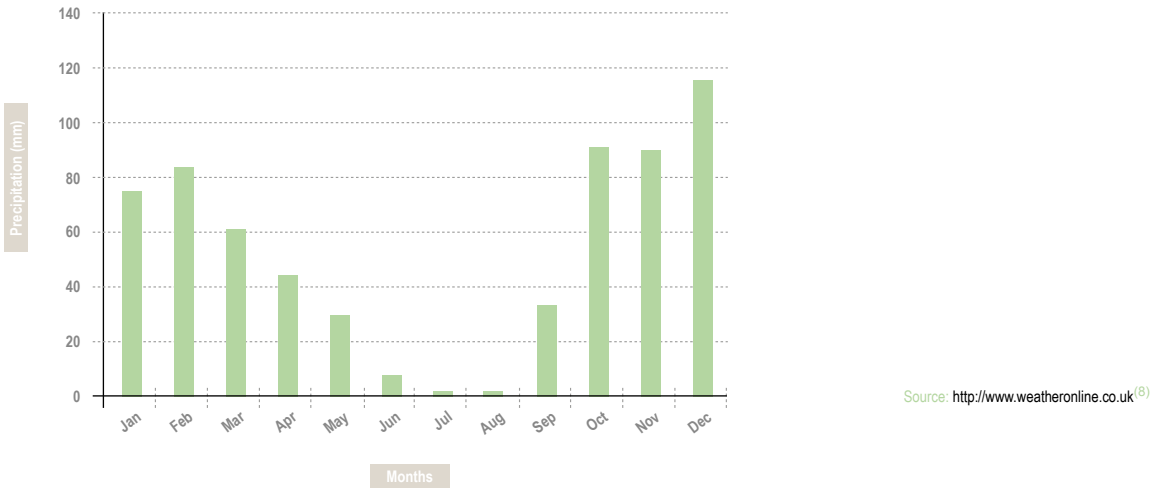
Figure:

① Minimum and maximum average temperatures (°C) in Funchal (Madeira) for each month of the year

Source: http://www.weatheronline.co.uk[8]

Figure:

② Precipitation (mm), in Funchal (Madeira), for each month of the year

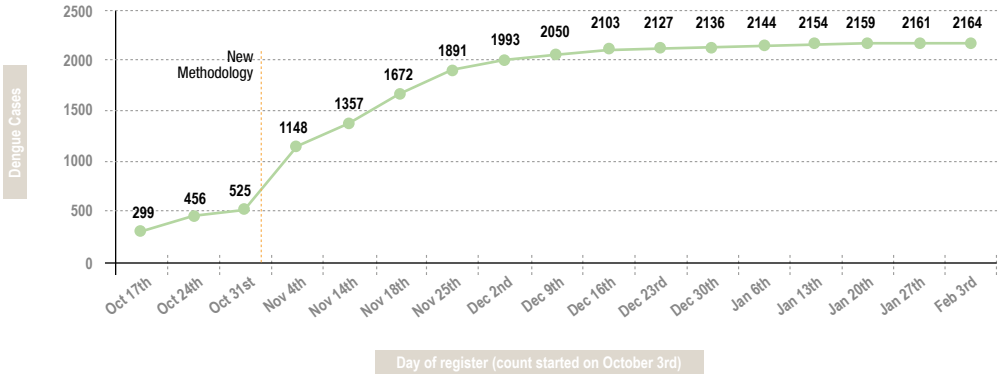


Source: http://www.weatheronline.co.uk[8]

The mosquito *Aedes Aegypti* was first identified in Madeira back in 2005, in the city of Funchal, and the dengue epidemic was officially declared by the Portuguese Health Authorities on the 3rd October of 2012 with two dengue confirmed cases. The evolution of Madeira's dengue fever, by week, is represented in ***Figure 3***[9], with the rate of dengue incidence decreasing throughout this time period.

Figure:

③ Cumulative evolution of the dengue fever in Madeira, from the 3rd of October (2012) to the 3rd of February (2013)



Source: Directorate-General of Health. New methodology - refers to alteration of case definition made by guidelines update [10]

Dengue cases can be classified as probable or confirmed. A case is considered probable when it meets both clinical and epidemiological criteria. A clinical criterion is characterized by fever and two or more of the following symptoms: headache, retro-orbital pain, myalgia, arthralgia, exanthema, hemorrhagic manifestations or leucopoenia. The epidemiological criterion is present when a person lives in or has been in the dengue affected area within a period of 21 days from the onset of symptoms. A confirmed case appears with one or more of the following laboratory results: i) the presence of dengue-virus-specific IgM antibodies in the blood or in the cerebrospinal fluid; ii) a significant increase in the concentration of dengue-virus-specific IgG antibodies, or; iii) the detection of the dengue virus' nucleic acid in blood[11].

Through this work, a greater understanding of the factors contributing to the dengue outbreak can be achieved, thus allowing health care planning better suited to the needs of the Madeira population.

The goal of this study was to better understand the evolution of the dengue fever in Madeira, attempting to anticipate its trend for the rest of the year 2013. For this, it was not possible to use past information as Madeira is experiencing a dengue epidemic for the first time.

② Methods

Being the first dengue epidemic in the archipelago of Madeira, there was no information about the disease to study its future evolution. Thus, information regarding outbreaks of this epidemic in other parts of the world was gathered to trace the behavior of the virus itself and to compare it to the case of Madeira. The selection of the countries followed some criteria to allow comparison with Madeira. Therefore, regions similar to Madeira were chosen, in terms of the conditions that are considered to foster the spread of dengue, namely the climate and the level of development.

For the first condition, countries with tropical and sub-tropical climates were chosen, with minimum and maximum temperatures of 15°C and 34°C, respectively. Countries with minimum average temperatures above 21°C were automatically excluded. At last, using this selection criterion, countries which had approximately the same number of rainy months as Madeira were selected.

The second condition, the level of development of a country, can often be evaluated through the following indicators: *infant*

mortality rate, urban population percentage and population density. Hence, considering Madeira's profile, this criterion would only include countries with an *infant mortality* rate less than or equal to 16 per million, a *percentage of urban population* between 60% and 90% and a *population density* between 100/km^2 and 450/km^2. Overall, this criterion cannot be too strict, as it is difficult for the countries to fulfill all of the requirements. Candidate countries were those which had previous dengue outbreaks. Afterwards, they were selected according to the criteria described above with a focus on those which fulfilled as many of the above requirements as possible.

The six countries selected were: Cuba, The Bahamas, Guadeloupe, Vietnam (in particular South Vietnam), Australia (in particular Queensland) and Puerto Rico.

After, it was given a deep look at their dengue trend along the history, especially at the first outbreak because Madeira is experiencing that same situation as well. When the information was only available in terms of absolute figures, the *attack rates* were estimated using the formula:

$$attack\ rates = \frac{number\ of\ dengue\ cases}{total\ population} * 100$$

However, these *attack rates* refer to the total population of the country. Thus, as the mosquito of dengue chooses preferably urban zones, the attack rate referring to the *urban population* was also estimated:

$$urban\ attack\ rates = \frac{number\ of\ dengue\ cases}{total\ population * \%\ urban\ population} * 100$$

It is also important to note whether those *attack rates* refer to the probable cases or to the laboratory-confirmed cases because the analysis has credibility only if one compares the same type of indicators. The frequency and the duration of the outbreaks were also registered and, whenever possible, the serotypes were identified.

After gathering the information about all of the dengue outbreaks, it was noticed that some of them happened a long time ago and, of course, the conditions of the countries were not exactly the same at that time. Hence, more information relative to those past decades was obtained to see if the countries, during those years, would compare to current Madeira.

3

Results

A total of six countries were selected: Cuba, The Bahamas, Guadeloupe, Vietnam (in particular South Vietnam), Australia (in particular Queensland) and Puerto Rico. They all have tropical or subtropical climates and their minimum and maximum temperatures do not exceed the interval from 15°C to 34°C, respectively. Furthermore, recall that Madeira has four particularly rainy months and so the selected countries have between four to seven months of high rainfall. In terms of *infant mortality* rate, all the countries have less than 16 per million, highlighting that the lowest and the highest cases are 3.9 per million in Australia (2011) and 15.8 per million in The Bahamas (2003), respectively. There is an exception in the case of Vietnam, year 2002: 30 per million. Regarding to the population, The Bahamas, Guadeloupe, Puerto Rico and Australia-Queensland, with between 0.3 and 4.5 million inhabitants, were the most similar to Madeira, which has 0.4 million inhabitants. On the other hand, the *population density* varies between 166/km^2 and 436/km^2, for Cuba (2002) and Guadeloupe (2002), respectively. Concerning to the *urban population*, its percentage varied between 66% in Cuba (1977) and 100% in The Bahamas (2011). Once again, Vietnam does not fulfill the requirement and the same goes for Guadeloupe. These countries present values of approximately 17% (1975), 24% (2002) and 28% (2009) for Vietnam and 48% (2002) for Guadeloupe. All the results are shown in *Table 1*.

Table:

① Resume of some of the characteristics of the six selected countries

Description of the Countries					
Country	Climate Min – Max Temp (°C) Rainy season **	Mortality Infant Rate (per million)*	Population (per million)*	Population density (/km2)*	Urban population (%)*
Cuba	Tropical 21°C-31°C [13] May to October	**6** (2002)	**9,6** (1977) [16] **11,3** (2002)	**166** (2002)	**66** (1977)[22] **75** (2002)
Guadeloupe	Subtropical tempered 20ºC-31°C July to November	**8,4** (2002) **6** (2009)	**0,4** (2002, 2005, 2009)	**436** (2002) **240** (2009)	**48** (2002) **100** (2011)
Puerto Rico	Tropical marine, mild 20°C-31°C April to November [14]	**10,6** (2002)	**2,3** (1963) [17] **3,9** (2002)	**698** (2002)	**48,7** (1963)[23] **67,4** (1981)[23] **95,8** (2002)[23]
Bahamas	Tropical Marine 15°C-31°C June to October	**15,8** (2003) **12** (2011)	**0,3** (2003) **0,4** (2011)	**36** (2003) **26** (2011)	**84** (2003, 2011)
Austrália	Tropical temperate 15°C-29°C [15] December to May[[15]	**5,2** (2009) **3,9** (2011)	**13,5** (1992) [18] **19,7** (2002) **22,7** (2011) **Queensland: 3** (1992) [19] **4** (2007-2010) [19] **4,5** (2011) [19]	**4** (2002) **3** (2011)	**85** (2002) **82** (2011)
Vietname	Tropical 15°C-34°C mid-May to mid-September	**30** (2002) **15** (2009)	**48** (1975)**79,7** (2002)**87,3** (2009) **South Vietname: 22** (1988)[20] **27** (1998) [21]	**389** (2002) **263** (2009)	**≈17** (1975) [24] **24** (2002) **28** (2009)

* *Source (unless otherwise specified):* **Population Reference Bureau, World Population Data Sheet** [4]
** *Source (unless otherwise specified):* **http://www.climate-zone.com/** [12]

It can be observed that those regions satisfy almost all the criteria (failing at maximum one), which allows a reliable comparison to Madeira. The exception is Vietnam, as it does not fulfill more than one of the requirements. Thus, this country was considered the "weakest" case, i.e., the least representative region in the study.

In *Table 2* is represented the dengue outbreaks' evolution along the past decades in the six selected countries.

Table:

② Dengue outbreaks' history in the six selected countries

Dengue outbreaks								
Region	Year	Serotype	Duration	Frequency	Type	Number of cases	Attack rate (%)	Urban attack rate (%)
Cuba	1977[25]	DEN-1[32]	2 years[25]	–	Clinical [25]	477440 cases (1977)[25] 75692 (1979)[25]	5 0,77	7,5 1,2
	981[26]	DEN-2[26]	ND	2 years	Clinical [26]	344203[26]	3,4	5,1
	1997[26]	DEN-2[26]	ND	16 years	Laboratory-confirmed [26]	2946[26]	0,03	0,04
	2001[26]	DEN-3[26]	ND	1 year	Clinical [26]	11432[26]	0,1	0,14
	2002[26]	ND	ND	1 year	Clinical [26]	3011[26]	0,03	0,04
Guadeloupe	2001[27]	ND	ND	4 years	Clinical [27]	2400	0,6 [27]	1,4
	2005[27]	DEN-4[27]	4-6 months[27]	4 years	Clinical [27]	11500[27]	2,9	4,4
	2007[27]	DEN-2[27]	4-6 months[27]	2 years	Clinical [27]	19000[27]	4,8	6,1
	2009[28]	ND	Almost 1 year[28]	2 years	Clinical [28]	41100[28]	10	11,3

Dengue outbreaks

Region	Year	Serotype	Duration	Frequency	Type	Number of cases	Attack rate (%)	Urban attack rate (%)
Puerto Rico	1963[25]	DEN-3[25]	1 year[25]	–	Laboratory-confirmed [25]	>27000[25]	1,2	2,5
	1972[25]	DEN-2[25]	1 year[25]	9 years	Clinical [25]	> 7000[25]	0,26	0,4
	1977[25]	DEN-1,2,3 [25]	ND	5 years	Clinical [25]	355000[25]	12,2	18,7
	1981[[26]	DEN-1[26]	2 years[26]	4 years	Clinical [26]	8350 (1981) 9536 (1982)[26]	0,28 0,32	0,4 0,5
	1986[26]	DEN-4[26]	ND	5 years	Clinical [26]	10659[26]	0,33	0,5
	1994[26]	DEN-2[26]	ND	8 years	Clinical [26]	22000[26]	0,63	0,76
	1997[26]	ND	ND	3 years	Clinical [26]	6955[26]	0,19	0,21
	1998[26]	ND	ND	1 years	Clinical [26]	17241[26]	0,47	0,5
	1999[26]	ND	ND	1 years	Clinical [26]	4993[26]	0,13	0,15
	2000[26]	ND	ND	1 years	Clinical [26]	2433[26]	0,06	0,07
	2001[26]	ND	ND	1 years	Clinical [26]	5233[26]	0,14	0,16
	2002[26]	ND	ND	1 years	Clinical [26]	1662[26]	0,04	0,06
Bahamas	1998[29]	DEN-1[29]	ND	–	Laboratory-confirmed [29]	365 [29]	0,15	0,18
	2003[29]	DEN-1[29]	ND	5 years	Laboratory-confirmed [29]	155[29]	0,05	0,06
	2011[29]	DEN-1[29]	ND	8 years	Laboratory-confirmed [29]	>3500[29]	0,88	1
Austrália*	1992[30]	DEN-2[30]	1 ano	–	Laboratory-confirmed [30]	≈20700	0,69[30]	0,78
	Past 5 years[30]	DEN-1,2,3 3[30]	ND	–	Laboratory-confirmed [30]	30-50 cases/ year[30]	0,001	0,002
Vietname**	1975[20]	ND	ND	–	Clinical [20]	19416[20]	0,11[20]	0,2
	1979[20]	ND	ND	4 years	Clinical [20]	21285[20]	0,12[20]	0,2
	1983[20]	ND	6 months[21]	4 years	Clinical [20]	77087[20]	0,38[20]	0,6
	1987[20]	DEN-2 DEN-3 [21]	6 months[21]	2 years	Clinical [20]	83905[20]	0,38[20]	0,55
	1988[20]	ND	ND	1 year	Clinical [20]	49237[20]	0,22[20]	0,3
	1992[20]	ND	ND	4 years	Clinical [20]	42363[20]	0,17[20]	0,93
	1997[21]	DEN-2[21]	6 months[21]	10 years	Clinical [21]	≈77000[21]	0,3	1,3
	1998[21]	DEN-3[21]	6 months[21]	1 year	Clinical [21]	119429[21]	0,44[21]	2
	2009 [21]	ND	1 year[21]	11 years	Clinical [31]	≈87300	0,1[31]	0,35

Values with grey color: Calculated manually taking into account the given information (formulas given in Methods)
*Specific area of Queensland ** Specific area of South Vietnam

On the general, the outbreaks lasted from 4 months to 2 years, with the most common duration period being from 4 to 6 months. Their frequency varied between 1 and 16 years, with the most common frequency being 2 years.

Cuba, with its first outbreak in 1977 and the last one in 2002, experienced *attack rates* from 0.03% (2002) to 5% (1977) and *urban attack rates* from 0.04% (2002) to 7.5% (1977). The cases were only confirmed in the laboratory in 1997, with an attack rate of 0.03% and an urban attack rate of 0.04%. This shows that the attack rate in Cuba decreased over time. In a different way, Guadeloupe had its major outbreaks from 2001 to 2009. The *attack rates*, regarding all clinical cases, varied from 0.6% (2001) to 10% (2009) and the *urban attack rates* varied from 1.4% (2001) to 11.3% (2009). Puerto Rico, on its turn, has many records of dengue. The first main one appeared in 1963 whilst the last main one was in 2002. The *attack rates* in this country varied from 0.04% (2002) to 12.2% (1977) and the *urban attack rates* varied from 0.06% (2002) to 18.7% (1977). The Bahamas and Australia-Queensland, on the other hand, presented their information in terms of laboratory-confirmed cases. The Bahamas had the lowest *attack rates*, varying from 0.05% (2003) to 0.88%

(2011). The corresponding *urban attack rates* varied from 0.06% (2003) to 1% (2011). According to the source, tourism was the responsible for the large attack rate increase in the outbreak of 2011 when compared to that of 2003[29]. Australia-Queensland experienced its first main dengue outbreak in 1992, with an attack rate of 0.69% and an urban attack rate of 0.78%. Since then, this region has had between 30 and 50 cases per year, which corresponds to an attack rate of 0.001%. Finally, South Vietnam experienced several outbreaks from 1975 to 2009. Specifically, the *attack rates*, all relative to clinical cases, varied from 0.1% (2009) to 0.44% (1998) and the *urban attack rates* varied from 0.2% (1975 and 1979) to 2% (1998).

Lastly, it was done an accurate analysis from all of the above information and studied all the *attack rates* and the *urban attack rates* for both clinical and laboratory-confirmed cases, whose corresponding mean, median and mode are shown in *Table 3.*

Table:

③ Analysis of the attack rates and the urban attack rates, for both clinical and laboratory-confirmed cases

	Clinical Cases		Laboratory-Confirmed cases	
	Attack rates	Urban attack rates	Attack rates	Urban attack rates
Mean	0,8	1,3	0,4	0,7
Median	0,29	0,5	0,15	0,18
Mode	0,1	0,5	–	–

The *attack rates* and the *urban attack rates* of Guadeloupe in 2009 (10% and 11.3%, respectively) and Puerto Rico in 1977 (12.2% and 18.7%, respectively) were excluded from the above calculation because they were considered outliers. An external factor could have been the cause for these uncommon *attack rates* for Guadeloupe and Puerto Rico, since these extreme values only appear in one year.

From this table, it can be concluded that the dengue *attack rates* in Madeira were expected to be approximately from 0.8% to 1.3% (*urban population*), for clinical cases, and from around 0.4% (*urban population*) to 0.7%, for confirmed cases. Applying this attack rate to the population of 0.27 million living in Madeira Island, and taking into account that 0.19 million is *urban population*, a number between **2160 and 2470 for dengue clinical cases and between 760 and 1890 for dengue laboratory-confirmed** cases were expected.

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Discussion/Conclusion

In the archipelago of Madeira were diagnosed 2164 clinical cases until the 3rd of February of 2013. Since Madeira's rainy months are from November to February, this number is not likely to increase significantly for the rest of the year. In this study it was estimated a number between 2160 and 2470 dengue clinical cases to occur in 2013 so, for the moment, available data seems to be in close support of these results.

There was no reference to any connection between attack rates and frequency of outbreaks.

It can also be discussed the extension in which Madeira should be compared with the selected regions in terms of other indicators besides the climate and the level of development. Even so, the level of development was considered a very important indicator because it is a crucial factor for the spread of the disease. It is known that developed countries typically divulge more information

to their citizens and also have better means to fight diseases. The *infant mortality* rate is also generally considered a good measure of the population health[33] and, hence, is a good measure of the level of development of a country. Furthermore, the percentage of *urban population* and *population density* are also related to the spread of dengue, as urbanized and highly populated zones are the best places for the mosquitoes to thrive.

Tourism may be another factor influencing the spread of dengue because travelers can be good vectors of various diseases. When this factor was analyzed in the six chosen regions[34], it was concluded that Guadeloupe, Puerto Rico and The Bahamas were the most similar to Madeira. The mean *attack rates* for these regions were calculated again and similar results to the ones before were obtained. Thus, analyze the dengue history related to tourism does not add much information to the above study.

In addition, this analysis is subjective because the information about dengue past episodes in other countries is being studied and used to anticipate its intensity in Madeira and each country reacts to an outbreak in a different way. However, stressing that the goal of this study was not to get exact numbers of dengue cases in 2013, but to find some boundaries for them, it proves to be adequate. In fact, the prediction of the exact number of cases is unrealistic and the presentation of an estimate is the most useful tool for the Portuguese health institutions to help in the design of a mitigation plan and take action.

This work is just a simple and initial useful approach. Deeper and more complex analysis must be performed as the definitive information of this dengue epidemic in Madeira (2013) becomes gradually available.

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