RIASE
REVISTA IBERO-AMERICANA DE SAÚDE E ENVELHECIMENTO
REVISTA IBERO-AMERICANA DE SALUD Y ENVEJECIMIENTO

INVASIVE MOSQUITOES IN EUROPE AND IMPORTANCE OF THEIR SURVEILLANCE IN PORTUGAL

Márcia Alexandra da Silva Marques – Master in Quality and Environmental Management. Superior Diagnostic and Therapeutic Technique - Environmental Health in the Public Health Unit of the Health Centers Group of Alentejo Central of the Regional Health Administration of Alentejo. Portugal

Maria João Alves – Assistant Researcher at the National Institute of Health Dr. Ricardo Jorge. PhD in Biology. Master in Biotechnology. Graduated in biology

João Manuel Bernardo – Teacher at the Department of Landscape, Environment and Planning at the University of Évora. PhD in Ecology. Graduated in Biology

VOL. 3 N.º 3 DECEMBER 2017

ABSTRACT

Objective: To analyze the invasive process of mosquito species that represent a greater risk to Public Health in Portugal and Europe, its distribution and behavior in face of environmental conditions and to evaluate the importance of the existence of surveillance programs.

Methods: Data at European level were obtained from reports and risk assessments. At the national level, a questionnaire was used, based on data from the REVIVE Program and the Portuguese Institute of the Sea and Atmosphere and the SPSS program, version 21.

Results: The data reveal adequate climatic conditions in Portugal for the introduction of invasive species that are vectors of disease, as has happened in some European countries and as occurred with the occurrence of *Aedes aegypti* in Madeira in 2012.

Conclusions: mosquitoes that represent the greatest risk to public health in Portugal and Europe are *Aedes aegypti* and *Aedes albopictus*. In Madeira, once the vector and the pathogen were introduced, climatic conditions favored the occurrence of Dengue cases. On the other hand, it is not clear responsibility for control when disease or discomfort occurs in Portugal.

Keywords: Vector-borne diseases; *Aedes aegypti*; *Aedes albopictus*; mosquitoes.

INTRODUCTION

Mosquitoes play important roles in many ecosystems. However, they can be important vectors of pathogenic microorganisms causing harm to human health. Throughout history, many millions of cases of diseases transmitted by these vectors have been reported, being attributed many deaths and even more cases of morbidity[1,2].

Tropical regions are the hardest hit. However, the increase in the dispersion of the species of mosquito vectors, primarily due to the increased movement of people and goods leads to increased risks to other regions[3,4].

The probability of survival and the subsequent establishment of invasive species in different geographical areas increase if the ecological conditions are favorable to them. Mosquitoes are not adapted to very low temperatures or very high temperatures. Thus, if climate change leads to an increase in minimum temperatures, it can increase the risk of dispersion[5].

In Europe, the species considered to be the greatest threats to public health are *Aedes aegypti* and *Aedes albopictus*, has already been responsible for several cases of disease[6].

In Portugal, the date of preparation of this work was not yet detected the presence of *Aedes albopictus*, but *Aedes aegypti* is present on the island of Madeira and caused a Dengue outbreak in 2012.

This paper reviews the literature on mosquitoes that are relevant in terms of public health in Europe and Portugal, explaining their presence and impacts, emphasizing the most significant aspects and the importance of their surveillance in our country. Thus, the objectives are: to identify the fauna of invading disease-causing mosquitoes in Europe; identify the risk associated with causing mosquito disease in Portugal; understand whether climate change may contribute to the dispersion of invading mosquitoes; to determine the influence of environmental factors on the ecology, development and survival of invasive mosquitoes causing disease; evaluate the importance of the existence of surveillance programs and identify forms of intervention.

MATERIAL AND METHODS

Data at European level, were obtained in reports and risk assessments from European Center for Disease Prevention and Control (ECDC) and the European Network for Arthropod Vector Surveillance for Human Public Health (VBORNET).

In the analysis of the occurrence of *Aedes aegypti* in Portugal and in relation to climatic factors and other species, we used data from the REVIVE Program and the Portuguese Institute of the Sea and Atmosphere and the SPSS Program, version 21.

In the identification of forms of action in relation to occurrences of situations related to mosquitoes in the last ten years, a questionnaire was sent to the Regional Health Administrations. Four completed questionnaires were collected namely from the ARS Center, Lisboa e Vale do Tejo, Alentejo and Algarve.

RESULTS

Mosquitoes live in a wide variety of habitats, except for those that are permanently frozen, and have been increasingly distributed throughout the world in recent decades. Several factors have contributed to the dispersion of mosquitoes. The increase in global trade and the migration of human beings are the main causes of the dispersion of mosquitoes, while extending their habitats of forests to urban environments. Some species show great plasticity[6].

Although mosquito-borne diseases are much more prevalent in tropical than temperate regions, due to favorable vector climatic conditions in these areas, there have always been endemic and autochthonous epidemics in Europe. However, the worry is increasing because vectors and pathogens are increasingly being introduced into new areas.

Some vector-borne diseases are emerging, or reappearing after long absences, while others are spreading. Its occurrence is often associated with changes in ecosystems, human behavior and climate.

This is illustrated by the recent outbreaks of *Chikungunya* and *West Nile*[4,7].

In Portugal, a key to identify mosquitoes from mainland Portugal, the Azores and Madeira was published in 1999, with 45 being the number of species identified[8].

In order to know exactly which vector species are present in Portugal, in what regions and what their vectorial capacity, a Vector Surveillance Network was created, called REVIVE, at national level, resulting from a Protocol between the General Direction of Health, Institute of Health Administration and Social Affairs, IP- Região Autónoma da Madeira and the Center for the Study of Vectors and Infectious Diseases Dr. Francisco Cambournac/National Institute of Health Dr. Ricardo Jorge[9].

Between 2008 and 2012 the REVIVE identified 25 species belonging to thoe mosquito fauna of Portugal, including *Aedes aegypti*, identified for the first time in Madeira in 2005[10]. No invasive species were found, except for *Aedes aegypti* identified in Madeira. In neither case human pathogenic flaviviruses was identified[11]. In 2005 *Aedes aegypti* was detected in Madeira, and in October 2012 an outbreak of Dengue occurred in the archipelago. *Aedes aegypti*, absent from mainland Portugal since 1956, is currently reason of concern by the health authorities, due to the probability of reintroduction on the continent[9].

Oh the most abundant species in Portugal, two deserve special attention: *Culex pipiens* and *Culex theileri* both reservoirs and vectors of *West Nile* virus and both already responsible for disease transmission in Portugal. *West Nile* virus was isolated in *Culex pipiens* in the Algarve and *Culex theileri* was already a vector of Filariosis in Alcácer do Sal[12].

The temperature is an important factor in both the vector density and in its vector capabilities, increasing or decreasing the vector survival, affecting the growth rate of their populations, interfering with their susceptibility to pathogens, changing the agent of the incubation period vector and changing the activity and standard transmission between stations[13].

Increasing the temperature of the water in the breeding, the larval-adult transformation occurs more quickly, speeding up the reproductive cycle. By decreasing the time required for maturation, the size of the larvae is reduced and, consequently, smaller adults are formed[13]. The temperature limits for disease transmission by mosquitoes are 14-18°C at the lower limit and 35-40°C at the upper limit. A small increase in the lower limit could lead to an increase in disease transmission. However, an increase in the upper limit would lead to the death of the mosquito[13].

Climate affects the geographical and temporal distribution of disease vectors representing a major threat to health security[6,14]. Vector-borne diseases often exhibit distinct seasonal patterns, showing that the ecology, development, behavior and survival of mosquitoes and the dynamics of disease transmission are influenced by climatic factors. The same factors also play a crucial role in the survival and transmission of the pathogens they transmit[15]. Countries with a temperate climate, such as Portugal, run the risk of future climatic conditions being more favorable to vector-borne diseases due to climate change[16].

The climate change scenarios set for our country indicate an increase in the number of days with temperatures suitable for the transmission of malaria, so the risk of contracting disease in Portugal, in the presence of infected mosquitoes, would go from very low to medium. The mosquito vector of dengue, *Aedes* is also highly sensitive to climatic conditions. Several studies suggest that climate change can expose more than two billion people to Dengue transmission by 2080[14]. At this time there is no risk of contracting Dengue in Portugal since the vector has not been identified. However, if there were vector introduction the risk would be low. The climate change scenarios point to an increase in the number of days adequate for virus transmission, leading to an increase in their distribution, so that the risk of contracting the disease would become medium.

The *West Nile* virus has, in the present circumstances, a low risk in our country, despite the presence of competent vectors (various species of *Anopheles* and *Culex*) and viruses (in vectors, animals and humans). In scenarios of climate change in Portugal, periods of survival of mosquito vectors tend to increase, which could lead to an increasing number of hosts and consequent increased risk of contracting the disease to medium level.

In Europe there have been records of various diseases transmitted by mosquitoes. Dengue, *Chikungunya*, *West Nile*, Malaria and Filariasis are the most important. Table 1 shows the relationship of these diseases with their transmission in Europe and the vector mosquito[6].

Table 1 – Relationship between Malaria, Dengue, Chikungunya, West Nile Virus and Filariasis, with its distribution in Europe and the vector mosquito.

Disease	Transmission in Europe	Vectors
Malaria	Endemic until the middle of th 20th century, since then only esporadic cases. Epidemic in greece in 2011.	*Anopheles spp.*
Dengue	Until the beginning of the 20th century; Croatia and France.	*Aedes aegypti, Aedes albopictus.*
West Nile Virus	Endemic in Southern Europe.	*Cx. pipiens, Cx. modestus, Aedes japonicus, Aedes atropalpus, Aedes albopictus, Ochlerotatus caspius, Aedes cinereus, Aedes vexans, Anopheles maculipennis.*
Chikungunya	Italy 2007; France 2010.	*Aedes aegypti, Aedes albopictus.*
Filariosis	Isolated cases especially in France, Italy and Spain.	*Ochlerotatus caspius, Aedes vexans, Culex theileri.*

Source: Adapted from World Health Organization/European Mosquito Control Association. *Guidelines for the control of invasive mosquitoes and vector-borne diseases in Europe 2011.*

The vector-borne diseases are a specific group of infections that pose a threat to Europe and require surveillance of exotic mosquito species such as *Aedes albopictus*, *Aedes aegypti*, *Aedes atropalpus*, *Aedes japonicus*, *Aedes koreicus* and *Aedes triseriatus*[17].

The most important vector for Public Health in Europe is *Aedes albopictus*, due to its expansion and vector competence. It was first identified in 1979 in Albania. Colonization in Europe continued in Italy in 1990 and gradually spread to other countries (Figure 1) such as France, Greece, Spain, Slovenia and Albania, and is also present in southern Switzerland and is sporadically identified in Germany. This expansion continues to occur, which represents a serious risk to Public Health. *Aedes albopictus* was the vector responsible for a *Chikungunya* epidemic in 2007, near Ravenna, Italy. In 2010 he was responsible for registering cases of Dengue in Marseille and Croatia and two cases of *Chikungunya* in France[6,17].

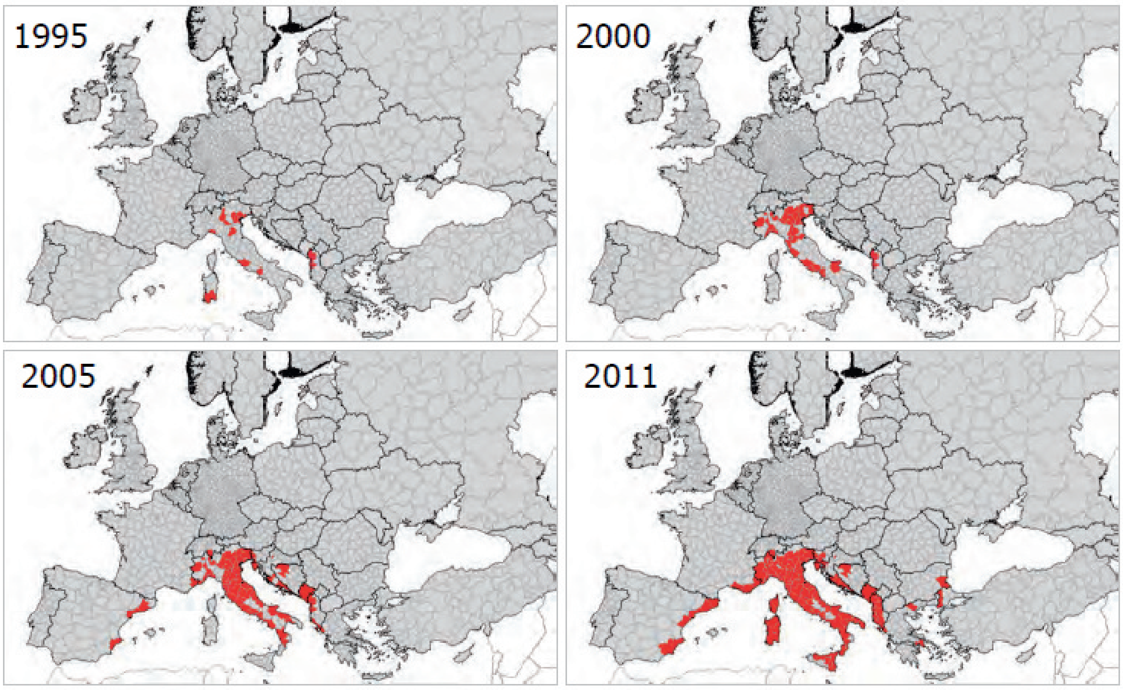

Figure 1 – Distribution of *Aedes albopictus* in Europe, 1995-2011.
Source: Print Screen of Guidelines for the surveillance of invasive mosquitoes in Europe, European Centre for Disease Prevention and Control, 2012.

Risk maps developed by Benedict *et al.* (2007) and ECDC (2009) predicted an expansion of *Aedes albopictus* by Europe, especially along the Mediterranean basin, which was verified, as can be seen in Figure 2.

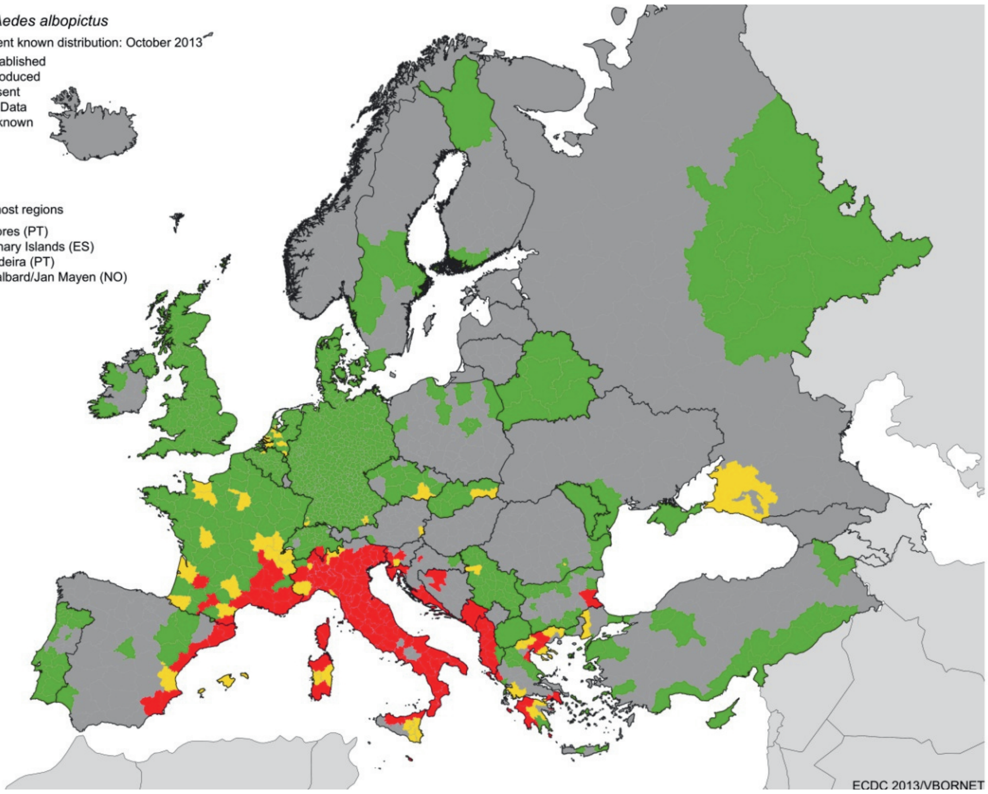

Figure 2 – Distribution of *Aedes albopictus* in Europe, 2013.
Source: European Center for Disease Prevention and Control/Vbornet, 2013. Available at: http://www.ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx. Consultation on 25-02-2014.

Another important vector is *Aedes aegypti*, which had disappeared from Europe in the last 50 years, was recently reintroduced around the Black Sea in southern Russia, Abkhazia, Georgia and Portugal in Madeira (Figure 3), increasing the concern of the Health Authorities. The high number of flights between the European continent and Madeira increases the likelihood of reintroduction of this species[6,20].

Figure 3 – Distribution of *Aedes aegypti* in Europe, 2013.
Source: European Center for Disease Prevention and Control/Vbornet, 2013. Available at: http://www.ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx. Consultation on 25-02-2014.

In Portugal, besides the risk of reintroduction in mainland of *Aedes aegypti*, there is still the risk of introduction of *Aedes albopictus*. Portugal is presented as one of the European countries with a high probability of installing this vector, not only because of climatic conditions but also because of human migrations and commercial traffic[17,19].

The authors used a genetic algorithm to determine the ecological niche of *Aedes albopictus* and to provide a global ecological risk map of species propagation. They combined this analysis with the risk of importing tires from infested countries and their proximity to countries that have already been invaded to develop a list of countries at greatest risk of establishment (Figure 4)[19].

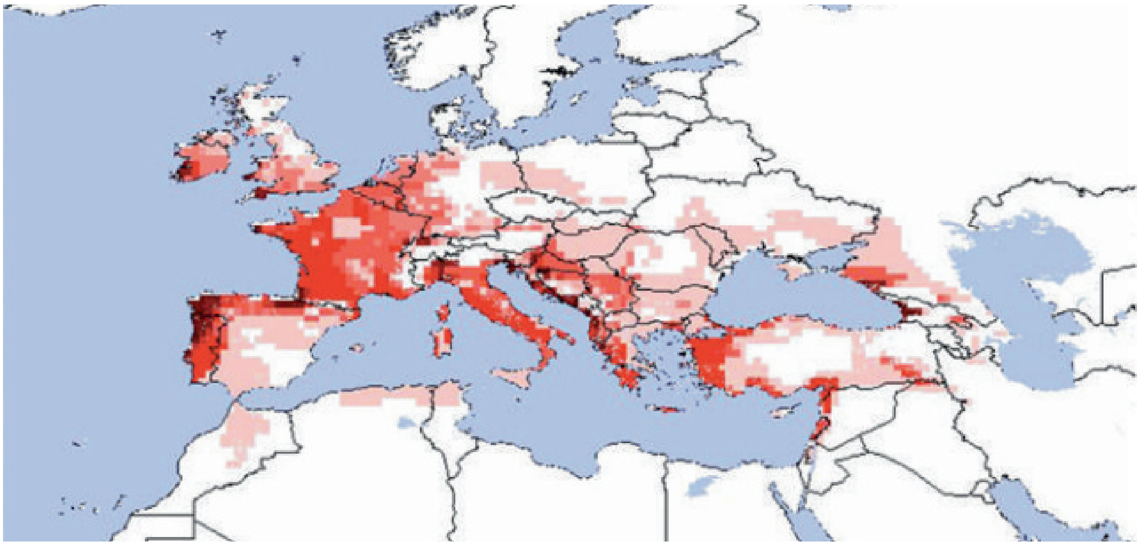

Figure 4 – Prediction of distribution potential of *Aedes albopictus* (darker shades indicate a greater number of models for suitable habitat).
Source: BENEDICT *et al.* (2007).

According to this study, the northern coast of our country is the area that has the highest risk of establishment. The environmental conditions considered were: the mean winter minimum temperature, above 0°C, which allows hibernation of eggs; the mean annual precipitation of 500 mm above, some precipitation in the summer and the mean summer temperatures above 20°C to allow their active dispersion.

The introduction of new invasive species is a worry. *Aedes albopictus* is found in many European countries. According to the risk maps published by this species, once introduced, it is very likely to settle in our country. This species is a great vector of dengue and a competent vector of over 20 arboviruses with potential impact on human health, including yellow fever, *West Nile* and *Chikungunya*(6,17,19).

Aedes aegypti was registered in the Autonomous Region of Madeira, for the first time in 2004-2005(10). In previous studies carried out between 1977 and 1979, this species had not been identified(12).

In 2005, the population of Santa Luzia, city of Funchal, began to to complain from the bites of an aggressive mosquito and were realized crops which led to identification of *Aedes aegypti*(10). In 2006 the species was for six neighborhoods in the city of Funchal.

Control actions have been taken such as reducing breeding sites, treatments with insecticides and education campaigns among the population, promoting individual protection and breeding reduction. However, the mosquito population persisted(12).

On October 3, 2012, two cases of Dengue virus infection on Madeira Island were confirmed at the CEVDI/INSA laboratory. On October 10, 18 more cases and 191 probable cases of Dengue were confirmed(21).

Since the beginning of the outbreak, October 3, 2012, until the day September 1, 2013 (latest data released by the DGS, the September 12, 2013) has been reported 2187 probable cases of Dengue fever, without any death. The number of Dengue cases with laboratorial confirmation declined steadily from mid-November 2012, and the outbreak was considered controlled on 3 March 2013. After that date, none of the cases identified was originated in Madeira Island(22,23).

The tests performed by the National Institute of Health identified the Dengue Virus Serotype 1 (DENV-1) with Latin American origin (Alves et al, 2013).

The ECDC's risk assessment concluded that this outbreak of Dengue fever in Madeira was a significant public health event due not only to the local population but also to the large number of tourists in the Madeira archipelago(24).

Based on data from the REVIVE program, a sample of 366 adult mosquitoes crop in two municipalities and 144 immature crops was analyzed in four municipalities in 2010, 2011 and 2012. The species with the highest average number of individuals per crop was *Aedes aegypti*.

The level of occurrence of *Aedes aegypti* adult mosquitoes were positively related to the minimum temperature, ie, increased with increasing the minimum temperature and with the minimum relative humidity values recorded during the harvest. Thus, their occurrence increases when there is an increase in the minimum relative humidity.

The main objective of epidemiological surveillance is the early detection of cases of introduction or alteration of mosquito species abundances, so that control measures are indicated in a timely manner in order to avoid new occurrences, thus preventing epidemics. Surveillance includes the data picking systematic and continuous, notification of confirmed cases, monitoring the distribution, dissemination and severity of cases, determination of geographical distribution and vector density, evaluation of the effectiveness of prevention and control programs, identification of risk areas and interpretation and dissemination of data.

The European Center for Disease Prevention and Control (ECDC) was established in 2005. It is an EU agency based in Stockholm, Sweden, with the aim of strengthening Europe's defenses against infectious diseases. ECDC is the entity responsible for the identification, assessment and reporting of infectious disease threats to human health.

ECDC has developed the Program on emerging and vector-borne diseases and a network of vector surveillance across Europe (VBORNET) for which Portugal collaborates through the REVIVE program.

Portugal (mainland) is one of the countries that, although it does not have invasive mosquitoes, have climatic conditions that allow the survival and proliferation of its eggs, so, according to the ECDC Guidelines, it must have surveillance programs. In the archipelago of Madeira, once the *Aedes aegypti* vector is established, it must be accompanied by its seasonal dynamics and monitored commercial routes, to prevent their expansion.

The International Health Regulations (IHR) establishes procedures to prevent the spread of diseases at international level, through monitoring, surveillance and response to emergencies in Public Health. Thus, according to the IHR, the State should be equipped with programs and trained people to control vectors and reservoirs at their points of entry (ports, airports and borders).

According to the results of the REVIVE Surveillance Program, no invasive species was found at the date of this study except for *Aedes aegypti* identified in Madeira. In no case were flaviviruses pathogenic to humans[25].

In order to understand how it is made to management and control, during events related to mosquitoes in Portugal questionnaires were sent to the Regional Health Administrations. Were recorded ten occurrences related to mosquitoes in the last ten years under the Regional Health Administrations, which resulted in two hospitalizations in the Alentejo, resulting from stinging and in two *West Nile virus* cases in the Algarve. The remaining situations refer to discomfort.

In some situations, the Health Authorities contacted the municipalities, in other situations they had the support of tour operators and the Public Health Department of the Algarve counted with the collaboration of public management entities in the environmental area such as APA-ARH and the CCDR (Regional Coordination and Development Commission) of the Algarve, in addition to the municipalities, for the implementation of control measures.

The information to the population seems to have been made with the support of the social media and some awareness actions in the room. In some cases the information focused on preventive measures (Alentejo), in others reassure of the population (Algarve) and in others only information on measures implemented (Lisbon).

CONCLUSIONS

Mosquitoes represent a public health problem when they occur in high densities, causing discomfort, or when they are vectors of disease, infecting people and animals with pathogens.

The mosquito species that represent the highest risk for Public Health in Portugal and Europe are *Aedes aegypti* and *Aedes albopictus*, and have already been responsible for several cases of disease. *Aedes aegypti* is part of the mainland Portugal mosquito fauna, although it has not been identified since 1956, and there is a risk of introduction of *Aedes albopictus*.

Of the most abundant autochthonous species in mainland Portugal, two are reservoirs and vectors of *West Nile* virus, *Culex pipiens* and *Culex theileri*(25), both already responsible for the transmission of disease in Portugal. The *West Nile virus* was isolated in *Culex pipiens* in the Algarve(26) and *Culex theileri* was already vector of Filariosis in the municipality of Alcácer do Sal(27). Serological tests on birds and horses reveal the presence and circulation of *West Nile virus* in Portugal(28).

Mosquitoes do not survive for long either at very low temperatures or at very high temperatures. The predictions of climate change point to an increase in temperatures. Rising maximum temperatures may increase species mortality, but raising the minimum temperature may lead to increases in density. On the other hand, a warmer atmosphere contains more humidity. Also the increase of precipitation can lead to the increase of the number of breeding places. The combination of these factors may be responsible for the increased risk of transmission of arboviruses in Portugal.

In the analysis of the relationship between climatic factors and dengue cases during the outbreak that began in Madeira in 2012, it can be concluded that, once the pathogen was introduced, climatic conditions favored the occurrence of Dengue cases. The three climatic factors considered (temperature, humidity and precipitation) provide favorable conditions for the development of the *Aedes aegypti* vector, which together with the increase of its density and the presence of the virus trigger the transmission of Dengue.

The results, in the analysis to REVIVE data 2010-2012, demonstrate that the occurrence of *Aedes aegypti* in Madeira was influenced by the climatic factors minimum temperature and minimum relative humidity.

It can also be concluded that Portugal does not have a guide document that establishes communication circuits, action strategies, responsibilities, control criteria and methods, collaboration strategies in information to the population, among others. Thus, we expect the publication by the DGS of a National Contingency Plan to clearly define responsibilities and action measures in case of vector-borne disease events in Portugal.

We suggest the development of a prevention plan aimed at both the prevention and control of vector populations, which although not infected, are present in our country and to prevent the installation of invasive species such as *Aedes aegypti* and *Aedes albopictus*.

BIBLIOGRAPHY

1. Fang, J. 2010. A world without mosquitoes. Revista Nature 466: 432-434.

2. Mole, B.M. 2013. Bedeviled by Dengue. Available from: www.the-scientist.com/?articles.view/articleNo/34434/title/Bedeviled-by-Dengue. Accessed on May 14, 2013.

3. Christophers, S.R. 1960. *Aedes aegypti*, the yellow fever mosquito. Cambridge University Press, Londres.

4. Marí, R.B.; PEYDRÓ, R.J. 2009. La creciente amenaza de las invasiones biológicas de mosquitos sobre la salud pública española. Enfermedades Emergentes 11(1): 30-35.

5. Epstein, P.R. *et al.*. 1998. Biological and physical signs of climate change: focus on mosquito-borne diseases. Bulletin of the American Meteorological Society 79 (3): 409-417.

6. World Health Organization (WHO)/ European mosquito control association (EMCA). 2011. Guidelines for the control of invasive mosquitoes and associated vector-borne diseases on the European continent. WHO, Alemanha.

7. European Centre for Disease Prevention and Control (ECDC). 2012. The climatic suitability for dengue transmission in continental Europe. ECDC, Stockholm.

8. Ribeiro, H.; Ramos, H.C. 1999. Identification keys of the mosquitoes of Continental Portugal, Açores and Madeira. European Mosquito Bulletin 3: 1-11.

9. Alves, M.J. *et al.*. 2010. Relatório REVIVE 2010 - Programa Nacional de Vigilância de Vectores Culicídeos. Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, IP), Lisboa.

10. Margarita, Y. *et al.*. 2006. Mosquitos de Portugal: primeiro registo de *Aedes* (Stegomia) aegypti Linnaeus, 1762 (Diptera, Culicidae) na Ilha da Madeira. Ata Parasitológica Portuguesa 13 (1): 59-61.

11. Alves, M.J. *et al.*. 2010. Relatório REVIVE 2008/2009 - Programa Nacional de Vigilância de Vectores Culicídeos. Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, IP), Lisboa.

12. Almeida, A.P.G. 2011. Os mosquitos (Diptera, Culicidae) e a sua importância médica em Portugal: desafios para o século XXI. Ata Médica Portuguesa 24 (6): 961-74.

13. López-Vélez, R.; Moreno R.M. 2005. Cambio climático en España y riesgo de enfermedades infecciosas y parasitarias transmitidas por artrópodos y roedores. Revista Española de Salud Pública 79 (2): 177-190.

14. World Health Organization (WHO)/ World Meteorological Organization (WMO). 2012. Atlas of health and Climate. Available from: http://www.who.int/globalchange/publications/atlas/report/en/index.html. Accessed on July 19, 2013.

15. Reiter, P. 2001. Climate change and mosquito-borne disease. Environmental health perspectives 109 (suppl 1), 141.

16. Santos, F. D. *et al.*. 2002. Climate Change in Portugal. Scenarios, Impacts and Adaptation Measures - SIAM Project. Gradiva, Lisboa.

17. European Centre for Disease Prevention and Control (ECDC). 2012. Guidelines for the surveillance of invasive mosquitoes in Europe. ECDC, Stockholm. Available from: http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf. Accessed on July 18, 2013.

18. European Centre for Disease Prevention and Control (ECDC). 2009. Development of *Aedes albopictus* risk maps. ECDC, Stockholm.

19. Benedict, M.Q. *et al.*. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector-Borne and Zoonotic Diseases 7(1): 76-85.

20. European Centre for Disease Prevention and Control (ECDC). 2012a. Autochthonous dengue cases in Madeira, Portugal. October 2012. ECDC, Stockholm.

21. Alves, M.J. *et al.*. 2013. Clinical presentation and laboratory findings for the first autochthonous cases of Dengue fever in Madeira island, Portugal, outubro 2012. Euro Surveill 18 (6) : 20398. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20398. Accessed on December 10, 2013.

22. Direcção Geral da Saúde (DGS). 2012. Dengue na Região Autónoma da Madeira – Comunicado da Direcção Geral de Saúde n.º C46.07.v1. DGS, Lisboa.

23. Direcção Geral da Saúde (DGS). 2013. Surto de dengue na Ilha da Madeira – situação em 19 de maio de 2013. DGS, Lisboa.

24. European Centre for Disease Prevention and Control (ECDC). 2013a. Dengue outbreak in Madeira, Portugal. October–November 2012. ECDC, Stockholm.

25. Alves, M.J. *et al.*. 2012. Relatório REVIVE 2012 - Programa Nacional de Vigilância de Vectores Culicídeos. Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, IP), Lisboa.

26. Connell, J. *et al.*. 2004. Two linked cases of *West Nile* vírus (WNV) acquired by Irish tourists in the Algarve, Portugal. Euro Surveill 8. Available from: http://www.eurosurveillance.org/ew/2004/040805.asp#1. Accessed on February 4, 2014

27. Esteves, *et al.* 2005. *West Nile* vírus in southern Portugal, 2004. Vector-borne and zoonotic Diseases 5 (4): 410-413.

28. Fevereiro, M. 2011. Resultados serológicos que demonstram a ocorrência de infecções pelo vírus West Nile (WNV) em equinos e aves em Portugal (2004-2011). Available from: http://dspace.uevora.pt/rdpc/handle/10174/4990. Accessed on February 4, 2014.

Correspondence: marquesmarci@gmail.com