# SCREENING OF MOSQUITOES AS VECTORS OF *FRANCISELLA TULARENSIS* IN PORTUGAL

CARINA LUÍSA CARVALHO [1], LÍBIA ZÉ-ZÉ [1], ELSA LECLERC DUARTE[2], MARIA SOFIA NÚNCIO[1], ISABEL LOPES DE CARVALHO[1]

[1]Center for Vectors and Infectious Diseases Research, National Institute of Health Dr. Ricardo Jorge, Águas de Moura, Portugal,

[2]ICAAM, University of Évora, Portugal

## Introduction

Tularemia is a zoonosis caused by *Francisella tularensis* that has recently emerged in new locations, populations and settings (1). This contagious septicemic disease affects mainly hares, sylvatic rabbits, rats, mice and other rodents. In some circumstances, the disease can also affect humans, domestic animals (herbivores and small carnivores), birds, fish and amphibians. The major route of infection is the skin by direct contact with dead or infected animals. Other routes of infection are the eye conjunctiva, mouth and nose mucous membrane (drinking contaminated water, ingestion of meat from sick animals or inhalation) or arthropod bites (2). The most important pathogenic subspecies are *F. tularensis* subsp. *holarctica* that occurs throughout the Northern hemisphere and *F. tularensis* subsp. *tularensis* that occurs usually in North America. Mosquitoes from genera *Culex* (Figure 1) and *Aedes* are considered important vectors for *F. tularensis,* especially in Sweden (3). In Portugal, there are 40 species of mosquitoes reported, being *Ochlerotatus caspius*, *Culex pipiens* and *Cx. theileri* the most frequent (4). *F. tularensis* subsp. *holarctica* was already detected in *Dermacentor reticulatus* ticks (1), however the role of mosquitoes remains unknown.

**Figure 1.** *Culex pipiens,* courtesy of Hugo Osório Center for Vectores and Infectious Diseases Research.

## Objectives

In this work, the role of mosquitoes in the transmission of *F. tularensis* in Portugal was investigated. We aimed to clarify if the species of mosquitoes reported in Portugal could act as competent vectors for *F. tularensis,* as reported in some European countries.

## Materials and Methods

An ongoing epidemiologic surveillance program on arthropod vectors (REVIVE) provided the samples that were analyzed in this study. A total of 4949 mosquitoes were investigated for the presence of *F. tularensis* of which 1373 (68 pools) were captured during the year of 2011 and 143 specimens were captured between 2007 and 2010, all over the national territory; 3433 mosquitoes (80 pools) were captured during the year of 2007 in the region of Algarve. The mosquitoes of this last group were collected in same year of the last outbreak in Spain. Pool mosquito samples were extracted using phenol:chloroform. Individual specimens DNA was extracted using DNeasy Blood and Tissue kit (Qiagen). A nested PCR for specific partial amplification of *tul4* gene was used for *F. tularensis* nucleic acid detection, as described by Karhukorpi and Karhukorpi (2001) (5).

## Results and Discussion

| Mosquito Genera | Percentage (%) | Mosquito Species | Results from PCR for *F. tularensis* detection (gene *tul4*) |
|---|---|---|---|
| *Culex* | 63.97 | *Cx. pipiens* | Negative |
| | | *Cx. theileri* | |
| | | *Cx. perexiguus* | |
| *Ochlerotatus* | 35.34 | *Oc. caspius* | Negative |
| | | *Oc. detritus* | |
| *Anopheles* | 0.42 | *An maculipennis* | Negative |
| *Culiseta* | 0.14 | *Cs. longiareolata* | Negative |
| | | *Cs. annulata* | |
| *Aedes* (*) | 0.12 | *A. aegypti* (*) | Negative |

**Table 1**: Mosquitoes studied for the presence of *F. tularensis.* (*) A small number of *Aedes aegypti* females from Madeira island were also analyzed.

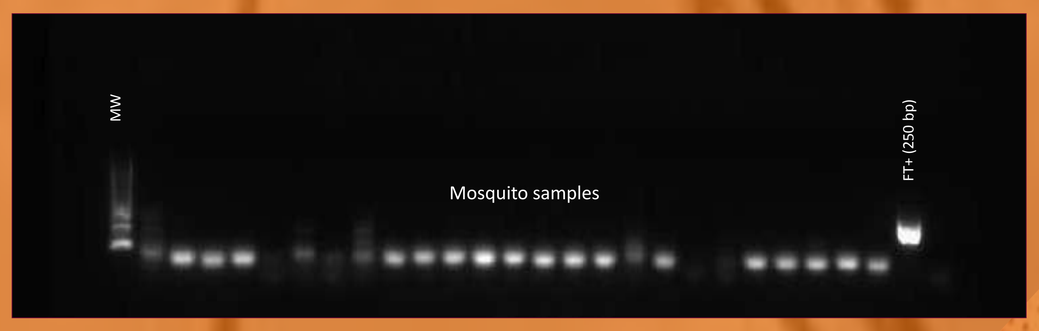


**Figure 2** : *Francisella tul4* negative amplification results from mosquito samples in 1.5% agarose gel. FT+: positive control; MW: DNA marker.

All samples investigated were negative for the presence of *F. tularensis* (Table 1 and Figure 2). These results suggest that in Portugal mosquitoes do not play a crucial role as vectors for *F. tularensis.* Ticks are probably the most important vectors for this pathogen as it happens in the majority of countries were tularemia is endemic.

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