Team's/Director's names: UMR INSERM 1094 Neuroépidémiologie Tropicale / Pr Preux

Precision on the supervision (name of the supervisor(s)): Thesis in co-direction Dr A Mercier (UMR INSERM 1094 – Limoges) & Pr ML Dardé (UMR INSERM 1094 – Limoges)

Keywords: *Toxoplasma gondii* – diversity – genomic introgression – molecular epidemiology– France – Africa

Profile and skills required: The candidate must have an academic background in life sciences (eg biology of organisms), have a good knowledge of population genetics and more largely in ecology. Expertise in epidemiology, parasitology, and molecular biology would also be appreciated. He or she must have a correct level of English and have a particular interest in the tropical environment, fieldwork and curiosity for zoology (animal behavior, species diversity).

Thesis subject title: Introgression phenomena in the study of genetic diversity of *Toxoplasma gondii* between Nouvelle Aquitaine region (France) and West and Central Africa: possible environmental and human influences

Description of the research subject: *Toxoplasma gondii* infection is a ubiquitous zoonosis that affects all warm-blooded species. It has a complex epidemiology due to the wide range of hosts and transmission pathways involved. Contamination occurs mainly by oral route: by ingestion of oocysts shed in the environment by felids (final hosts) or by consumption of cysts-contaminated tissues. In human, it is estimated that one quarter of the human population is infected. Although it is generally benign in immunocompetent persons, life-threatening cerebral and multivisceral forms, in addition to ocular forms, have been described. This clinical variability seems to be related to the patient's immune status, but also to the virulence genetic determinants of the parasite strain. Over the last twenty years, a highly contrasted geographic distribution of the parasite populations in terms of genetic diversity has been demonstrated around the world. Determinants of this spatial structure are still poorly understood. In comparison to Europe and North America, a huge genetic diversity was found in South America, with highly pathogenic and even life-threatening strains described [1, 2, 3]. Limited data is available about genetic diversity in Asia and Africa. However, the few studies conducted in Africa give insights of a high prevalence in human and animals, with an unusual severity of the human infection [4]: incidence of ocular toxoplasmosis in patients born in West Africa was 100 times higher than in patients born in Great-Britain irrespective of their ethnic origins [5]; severe clinical forms among immunocompetent patients have also been reported in Africa [6]. A number of studies showed a link between the clinical expression and the causal strain, especially in South America [7]. In a globalized world, understanding how the genetic diversity of the pathogen is structured becomes an emerging theme in the study of the toxoplasmosis epidemiology. It is an important public health issue and a priority to consider controlling this major parasitosis. Preliminary studies conducted by our team showed the role of human factors in the evolution of this diversity. A study conducted in French Guiana [8] shows that the environment anthropization results in changes in the parasite's local genetic diversity. It highlights the possibility of introgressions of wild strains (more genetically diverse and virulent for humans than anthropized strains) in the anthropized environment, causing potential risks for human health [3, 8]. These introgressions could favor the appearance of virulent recombinants. On a global scale, cases of severe toxoplasmosis, unusual among immunocompetent patients in Europe, were identified in metropolitan France following the consumption of horsemeat imported from the American continent [9, 10]. Trade globalization seems to create risk situations that are still unexplored and represent new challenges in human and animal health. The circulation of hosts, whether natural (bird migrations) or anthropogenic (transport of meat products, plants contaminated with oocysts, introduction of live animals such as cats or rodents) could be a source of introgression of *T. gondii* genotypes from one continent to another. These hypotheses suggested in the scientific literature remain to be demonstrated.

Thematic Area Context: Parasitology – Field work in tropical and temperate areas – Molecular epidemiology

Objectives: The aim of the IntroTox project is to assess the impact of human and environmental disseminations of *T. gondii* strains on its genetic and genomic diversity among the animal reservoirs and their implications in human epidemiology between Nouvelle Aquitaine region and West and Central Africa. These regions share a long history of commercial exchanges since the triangular trade period and important bird migratory corridors connect them. The extent of these exchanges and the diversity of flows observed between these two regions could thus favor the transfer of virulent genotypes from one region to another and the appearance of recombinant genotypes. In the context of health ecology, the specific objectives of this project will be: (i) to compare the genetic diversity of *T. gondii* populations in animals from France and West (Senegal and Benin) and Central (Gabon) Africa, (ii) to demonstrate gene flows and reconstruct the evolutionary history of *T. gondii* between these regions, (iii) to evaluate the virulence of isolated strains and (iv) to analyse genomic rearrangements (recombinations) within described genotypes in relation to their phenotypic expression (virulence in mice).

Methodology: *T. gondii* strains will be isolated in areas that could be "bridges" of genetic exchange between these regions, such as present and past (colonial period) port areas1 and migratory birds gathering areas2. Isolation of the parasite will be carried out from hearts and / or brains of domestic, peridomestic (rodent) and wild (small mammals and birds) seropositive animals, harvested opportunistically. Isolation will be done by inoculation of the parasite's tropism organs to laboratory mice. Data already exist for France (National Reference Center for Toxoplasmosis -CNR- and Center for *Toxoplasma* Biological Resources -CRB-) and Senegal (work of a thesis which ends in 2018), but will be supplemented by more specific sampling in areas of interest. Genotyping of the strains (or direct DNA) will be done by a multilocus analysis technique based on 15 microsatellite markers, developed in our laboratory and internationally recognized [11]. Population genetics analysis will be helpful to estimate gene flows between the different regions and environments studied. These gene flows will be considered in the light of available data on bird migration and the history of maritime traffic linking France to its former colonies in Sub-Saharan Africa since triangular trade to the present day (history, intensity and nature of trade). A comparison with the genetic data of a large number of strains from Portugal, South America and the Caribbean available at the CNR/CRB, will allow to evaluate the impact of these exchanges during the modern period on populations of *T. gondii*. This work will be carried out using genome-based phylogenetic analyzes of the strains obtained or already isolated. The virulence of the strains obtained will be characterized in mice during isolation by taking into account the dose-effect relationship of the inoculum by quantitative PCR. In parallel, the genotypes obtained will be confronted with all the genetic and clinical data collected throughout the world by our team, including strains isolated in patients from Africa through the CNR activities. The full sequencing of the obtained strains will allow demonstrating possible genomic introgressions and their impact on the phenotypic characteristics of the strains [12].

Expected results: This thesis project will provide useful data for the understanding of toxoplasmosis eco-epidemiology in different study areas (prevalence, identification of reservoirs, circulation of the parasite in the environment, risk factors). As far as possible, this work will tend towards a better appreciation of the risks for human health in the study regions, by comparing the collected data with the genetic and clinical data available for toxoplasmosis cases in humans. Ultimately, this thesis will contribute to the provision of data for risk assessment in human and animal health related to the genetic diversity of this parasite. It will allow the identification of entry routes and zones or environments favorable for the emergence of new genotypes in Nouvelle-Aquitaine region, thus generating knowledge in areas of research that have not yet been explored.

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