Title: Evolutionary modelling to understand the interactions between hard tick metabolism and *Babesia* development in its tick vector

Mots-clés : Babesia, Ixodes, ODE model, stochastic modelling, multi-scale modelling, in-silico evolution

Résumé du projet (max. 2 000 caractères)**.**

The world is witnessing the emergence and spread of several vector-borne diseases (VBDs) threatening animal and public health. Babesiosis is one of such tick-borne cosmopolitan parasitic diseases, caused by the infection with intraerythrocytic protozoan parasites of the *Babesia* genus after the bite of an infected ixodid tick. In Europe, recent publications confirm the increase in the annual incidence of babesiosis in both humans and animals, in parallel with an increase in the risk of exposure to hard ticks. *Babesia* parasites rely on their hard tick host to ensure their survival and transmission. The fitness traits associated to those mechanisms are key elements to understand *Babesia* epidemiology and virulence. While most research focus on the piroplasm-vertebrate host interaction, surprisingly little is known regarding how this piroplasm adapts to its arthropod vector. This project will explore the evolution of the time *Babesia* parasites require to develop within the vector with an individual-based model of piroplasmosis transmission that includes Ixodes tick metabolism. Specifically, we will model the influence of blood-feeding length and multiple meals on parasite development. We will profit from previous modelling using malaria-mosquito interaction to build a system of ordinary differential equations (ODE) that will consider both *Babesia*-specific variables associated with oocyst production and sporozoite development, and *Ixodes*-specific variables associated with meal number and frequency, and reproductive energy. Exhaustive bibliography research will allow the formulation of the conceptual framework, and the metabolic processes studied will be measured experimentally if needed. Parameter sweeps will be performed to assess the effect of different parameter values obtained. Finally, an individual-based model of *Babesia* transmission and/or sporogony will be proposed.

Contexte et motivation (max. 2 000 caractères)

Urbanization, climate and environmental changes, hastens the emergence of vector-borne diseases. The economic and social burdens of these diseases on individuals, households and economies are tremendous. Tick-borne babesiosis is one of such arthropod-borne cosmopolitan parasitic diseases. Many vertebrate species can be affected by this disease transmitted by the bite of ixodid ticks, and it is of great interest as emerging zoonoses.

This disease is responsible for a malaria-like clinical setting with haemolytic anaemia, which can progress to death if the animal is not diagnosed in time. In humans, the infection may produce symptoms such as jaundice, renal and respiratory failure. In more susceptible populations, such as the elderly, asplenic, or immunocompromised individuals, the disease can become severe and even life-threatening, despite setting up of a therapeutic protocol. In Europe, recent publications clearly show that the annual incidence of babesiosis is increasing in

both humans and animals, and that it is evolving in parallel with the increase in the incidence of other tick-borne diseases.

The development of *Babesia* organisms inside the vector is a decisive event, but information regarding the detailed lifecycle inside the tick remains scarce. While it is known that the parasite migrates towards the salivary glands to complete their sporogony, the potential targets for the development of transmission blocking strategies need to be unveiled. Recently, a theoretical model of *Plasmodium* transmission demonstrated that the study of the interaction between the parasite and the vector metabolism is crucial to understand the parasite's evolutionary strategies such as the manipulation of the female's metabolism to increase the number of blood meals, improving mosquito fertility and longevity and therefore increasing the odds for parasite transmission and dispersion. Similar mechanisms, such as the length of the blood feeding may influence *Babesia* development.

Objectifs scientifiques (max. 2 000 caractères)

The feeding activity of ixodid ticks includes one blood meal per stage before moulting. The female tick must accomplish its feeding to ensure oogenesis. At the same time, Babesia parasites have adapted their life cycle, so they overcome their sexual cycle right after tick engorgement, but they seem to stay in a quiescent stage after invading ovaries to ensure larvae infection. Babesia parasites are thought to interact non-competitively with their vector during the blood meal and all along stages development, including during transovarian transmission, as they scavenge the surplus internal resources only after reproductive investment is restrained and successful oviposition occurred. However, whether this symbiotic interaction is relevant for the natural tick feeding cycle remains unexplored and is difficult to study experimentally. A mathematical approach will allow us to understand the biological processes behind this single blood meal scenario in the female tick.

The aim of the project is to determine the influence of *Babesia* parasites on tick's feeding behaviour, as a strategy to ensure its own survival and dissemination. To do so, our strategy includes the assembly of all pertinent research about hard tick's metabolism (1), with a main interest in blood feeding behaviour and blood metabolism. This approach will allow the recognition of the metabolically induced resource allocation initiated by blood feeding, such as vitellogenesis (vitellogenin production) and detoxification (fat body production) processes, among others. When needed, we will confirm by transcriptomics the nutritional mechanism of interest in an *Ixodes ricinus* experimental model (2). Then, we will generate a mathematical model (3), using differential equations that will consider vector-specific compartments (e.g. blood digested during a blood meal), and individual-based models that could give us information regarding the effect of bloodmeal in parasite sporogony.

Méthodologie et calendrier (max. 4 500 caractères) ;

Tasks	Jan	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov	Dec

1.1 Bibliographic research											
1.2 identification of target metabolic processes											
2.1 Set-up of experimental tick model											
2.2 Transcriptomic analysis											
3.1 Mathematical analysis											
4. Interpretation of results											

Task 1. Bibliographic research and identification of target metabolic processes during blood meal in hard ticks

This project will explore the evolution of the time *Babesia* parasites require to develop within the vector with an individual-based model of piroplasmosis transmission that includes Ixodes tick metabolism. To obtain all exploitable information needed to develop the model, the candidate would search in bibliographic databases available online and by contacting partner laboratories working on hard tick physiology (Biology Centre of the Czech Academy of Sciences; BC CAS, Czech Republic). All the information gathered will be analysed in order to characterise blood meal parameters such as feeding length, speed of feeding, production of vitellogenesis promoters, blood digestion speed, duration of oviposition, among others. Piroplasm data such as gametogenesis, haemocoel invasion and glycolysis will be included.

Task 2. Experimental study in a *Ixodes ricinus* model

If needed, we will use an experimental model of artificial tick infection and feeding, this model has been developed by our research team and will allow us to validate the data that will be exploited for mathematical modelling. Ten to Twenty female ticks will be engorged by capillary feeding with bovine red blood cells infected with *Babesia divergens* merozoites. A group of ticks will be used as negative control and will be fed with non-infected erythrocytes. If needed, we will be able to use an infection model using *Babesia microti* – infected mice that will be used to feed the female ticks directly. In both cases, ticks will be monitored for survival and hatching up until 3 months and obtained larvae will be counted and tested for Babesia transmission.

Task 3. Mathematical modelling

Differential equations are the mathematical work horse tools to describe the time evolution of a system, be it physical, biological or abstract. The main difficulty here comes from the fact that we need to couple them to an individual-based model to simulate the action of the evolution on the relevant parameters of the model.

Specifically, we will model the influence of blood-feeding length and multiple meals on parasite development. We will profit from previous modelling using malaria-mosquito interaction to build a system of ordinary differential equations (ODE) that will consider both *Babesia*-specific variables associated with oocyst

production and sporozoite development, and *Ixodes*-specific variables associated with meal number and frequency, and reproductive energy. Exhaustive bibliography research will allow the formulation of the conceptual framework, and the metabolic processes studied will be measured experimentally if needed. Sensitivity analysis will be performed to assess the importance of the different parameter values. Finally, an individual-based model of *Babesia* transmission and/or sporogony will be developed to investigate the evolutionary dynamics of these parameters.

In collaboration with the Lifewire research team (Inria), we will use the BIOCHAM modelling software, which is based on a reaction rule formalism initially developed for the modelling of cellular processes (signalling, cell cycle, circadian clock, differentiation, etc.), but that can also be applied to multi-scale and multi-agent modelling and ecosystem modelling. BIOCHAM's key features include the ability to create stochastic (CTMC), discrete, and Boolean differential interpretations (ODE) of reaction models, as well as efficient parameter search methods that can align with observed behaviours formalized in temporal logic.

Positionnement du projet par rapport à l'initiative MOPGA (max. 2 000 caractères) ;

The candidate will develop its activity mainly at the MiTick team, Joint Research Unit BIPAR (ANSES/EnvA/Inrae). The MiTick team is a research structure internationally recognised by its excellence on tick physiology and tick-borne pathogens research. The team has level 1 and 2 laboratories equipped to perform cell culture, molecular biology, proteomics, confocal microscopy, flow cytometry, among other techniques. In addition, a tick facility for the mass rearing of *I. ricinus* colonies, is well established at the ANSES animal house, in association with the MiTick team. This team currently represents the only French team investigating using in vivo and in vitro tick models to understand *Babesia* development.

Mathematical modelling will be performed at the Lifeware project team (Inria, Institut Polytechnique de Paris). Lifeware aims at developing formal methods for understanding the cell machinery and establishing computational paradigms in cell biology. It is based on the vision of cells as machines, biochemical reaction networks as programs, and on the use of concepts and tools from computer science to master the complexity of cell processes.

Collaboration internationale et/ou retombées pour le pays de résidence ou d'origine du candidat (max. 2 000 caractères)