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Thesis project progress report

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Thesis title: Genetic structuration of tick Tunisian populations and effect of plant essential oils on symbiotic bacteria diversity.

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Introduction:

The tick-borne diseases of livestock constitute a complex of several infections caused by numerous etiological agents such as be protozoal, rickettsial, bacterial or viral; their single common feature is that they can all be transmitted by ticks. Babesiosis and theileriosis are the two main parasitic diseases whose pathogens are transmitted by ticks. These pathogens affect livestock, humans and rodents and many other mammals, causing serious economic losses on farms.

In the literature, numerous studies have reported that ticks also harbor symbiotes that are important contributors to their survival. However, this role remains not very well established for most symbiotes. On the other hand, some studies have shown the implication of symbiotes in the transmission of pathogens and in the physiology of ticks, particularly the reproduction. Indeed, it is known that ticks feed exclusively on blood (strict hematophagous), and this from the beginning of their life. This ultra-specialized diet is not without consequences because if the blood is a protein-rich food, it remains relatively poor in certain nutrients such as vitamins (especially vitamin B). In order to get around this problem, ticks harbor symbiotic bacteria which supply them with essential metabolites. These obligatory symbiotic bacteria are transmitted to the next generation vertically (maternally). Several of them are related to vertebrate pathogens such as *Coxiella*-like, Ricktssia-like and Francisella-like bacteria. These endosymbionts only infect ticks.

In Tunisia, little is known about the microbiota diversity of ticks. In this context, our study is focusing on the diversity of the microbiota associated with ticks as well as the potential antibacterial effects of some essential oils of aromatic plants on these symbiotes. The expected findings may provide valuable information in this field. Indeed, the targeted treatment of symbiotic bacteria with the use of essential oils could may constitute a new academic and technological concept for controlling tick

populations with the potential of opening the way to innovative alternatives to chemical acaricides for controlling ticks in full respect with the One Health and EcoHealth principles (absence of negative impacts for Human and Environmental health). To our knowledge, no study has been conducted using this approach aimed at controlling ticks through their microbiota using oil active biomolecules to reduce the transmission of pathogens.

Objectives:

- Study by NGS the diversity of *Hyalomma* ticks microbiota from different species, regions, stages, physiological states, wild or from colonies using a model major tick species well mastered in the laboratory of Parasitology of the ENMV Sidi Thabet, namely the tick Hyalomma scupense which is a vector of tropical theileriosis an economically important cattle disease affecting small holders in the NENA region.
- Identify the primary symbiotes.
- Assess the effects of selected essential oils on *Hyalomma* ticks and investigate their effects on their endosymbiotes (qPCR).

Expected final deliverables:

- more advanced knowledge on the function of primary symbionts of ticks and the effect and mode of action of plant essential oils,
- defined technological components of an experimental prototype (active ingredient, active doses, direct and indirect effects) applied to the control of tick populations.

Thesis project progress

During the first year of the thesis, we proceeded as follows:

- firstly, we carried out an in-depth bibliographic research, we targeted particularly the recent studies dealing with the microbiota of the ticks and especially on the primary symbionts and their implications in physiology and the survival of ticks. We have selected the compulsory symbiotic bacteria which will be the subject of our investigations in the treatment with essential oils. These are: Coxiella-like, Ricktssia-like and Francisella-like. These three endosymbionts play an essential role in the production of vitamin B (B12, B9, B2), nitrogen recycling and have a crucial role in the reproduction of ticks.

In addition, bibliographic research was conducted on the essential oil effects on both acaricide and antibacterial in order to select oils with a strong antibacterial acaricide activity at low doses. Thus, we have chosen to test 3 different essential oils from three aromatic plants possessing low active doses on ticks, these are namely peppermint, rosemary and thyme.

In a second part we carried out:

- the sampling of adults unfed H. scupense ticks in June 2019 (200 specimens) and the sampling of engorged nymphs (400) in October 2019, then we carried out their breeding, other Hyalomma species were also included in the study when present in the same farms or collected at the occasion of other surveys carried out at the ENMV Sidi Thabet.

- optimization of DNA extraction from ticks
- optimization of rRr16s PCR
- DNA extraction and PCR of rr16s from fasted adults
- DNA extraction and PCR of rr16s from nymphs.

DNA extraction and PCR will be followed by a microbiota diversity study by high throughput sequencing.

- The rest of our work will therefore consist of studying the diversity of the tick microbiota (genus *Hyalomma*) and exploring the acaricidal activity of essential oils on their endosymbionts to consider an alternative to control these pests.

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