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Progress report 2018

Phenology and phylogeny of *Hyalomma dromedarii* in the Sahara of South Tunisia and tick-borne pathogens in camels

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Cover photo—Herd of camels in Remada – South of Tunisia (photo credit: Ecole Nationale de Médecine Vétérinaire - Tunisia).

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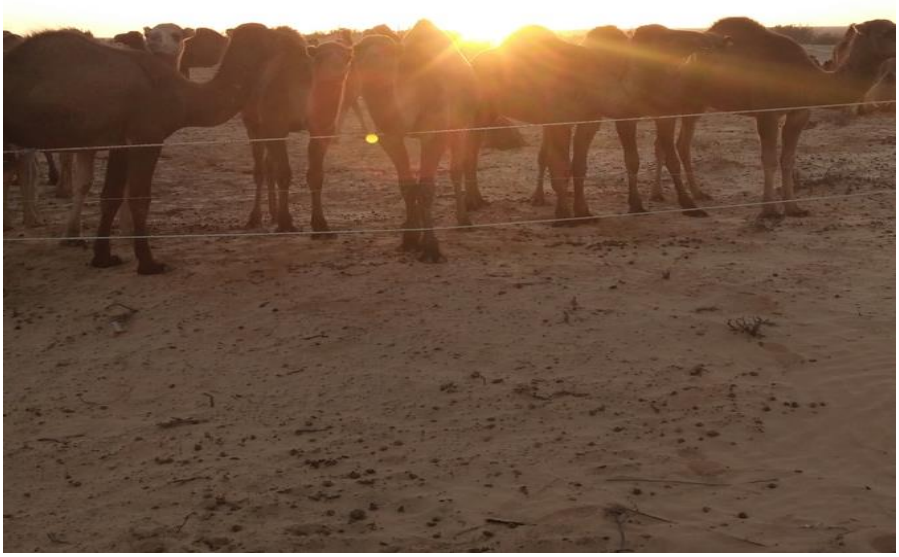

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Introduction/background

About 40% of Tunisia is occupied by the desert in the southern part. In the extreme south (Tataouine district), the climatic conditions are characterized by very low rainfall (variation between 1 and 75 mm), high temperatures (can reach 46°C) and frequent wind blowing (sirocco in summer). These conditions represent an important constraint to the development of livestock activities. Under these very harsh conditions, dromedary production is the dominant form of livestock particularly in the expanded communal grazing pastures such as Dhahar and El-Ouara. While goats are also important in the deep south of the country, dromedary production plays an important social, political and economic role for rural populations in Southern Tunisia. The camels' population (*Camelus dromedarius*) is estimated to 45,000 heads, almost a quarter of them (11000) are inventoried in Tataouine district (CRDA Tataouine, 2018).

Several constraints are hampering the management of dromedaries and their productivity. Camels face several diseases such as mange, surra, gastro-intestinal parasites, Q Fever (seroprevalence rate in Tunisia was estimated to 44%), ring worms, brucellosis and calf diarrhea with high mortality rates between 25-60%. These animals are particularly exposed to tick infestation and tick-borne pathogens.

During the last few years, several studies were conducted to investigate the zoonotic role and the diversity of Tick-borne Rickettsiae (Abdel-Shafy et al., 2012; Tomassone et al., 2016). The first detection of *Rickettsia aeschlimannii* was reported by Demoncheaux et al. (2012) in a very small number of ticks from camels under intensive breeding system (milk production under 0 grazing). There is limited data showing the real situation of camel infection with Spotted Fever Group *Rickettsiae* in Tunisia.

Moreover, previous studies provided strong evidence of antibodies circulation of Crimean Congo Hemorrhagic Fever virus in humans, cattle, sheep and ticks (Hyalomma *marginatum*) (Christova et al., 2018; Papa et al., 2017; Rodriguez et al., 1997; Wasfi et al., 2016). Epidemiological roles of *Hyalomma dromedarii* as a vector of several pathogens and the camels as a reservoir of numerous diseases are still unknown. The study of the epidemiology of these species needs to consider some aspects such as the movement of animals between regions and the collective use of of pastures with other herds. *Hyalomma dromedarii* is the main tick species infesting camels in Tunisia (Gharbi et al., 2013). Climate change is a key factor of the epidemiology of this tick species, as the case of several other vectors. The increase of temperature leads to the emergence of this tick in new geographic areas where it has never been reported before. Consequently, new host species such as cattle could be infested by *Hyalomma dromedarii* (Christova et al., 2018; Wasfi et al., 2016)

Further to the knowledge gap related to this particular species of tick, very little is known on the behaviour of free-living instars (place of oviposition of engorged females) that could be targeted by non-chemical control tools.

The general objectives of the current study were to study the biology of *Hyalomma dromedarii* in its natural environment and to survey the pathogens it transmits. In the first part, we were interested to investigate the activity dynamics of *H. dromedarii* and the characterization of the abiotic factors where free-living specimens were collected. Then the life cycle of *H. dromedarii* in natural conditions will be established and the phylogenetic analyses of the tick population will be conducted. The second part of this survey aimed to perform a survey to test for circulation of Crimean Congo Hemorrhagic Fever virus in camels and ticks in southern Tunisia, and to determine the vectorial capacity of *Hyalomma dromedarii*. The molecular prevalence of *Rickettsia* associated with *Hyalomma* ticks will be

estimated. This report presents preliminary results for the survey conducted during the period between April and December 2018. More sampling rounds are scheduled for 2019 and additional secondary data will be collected to better characterize the working environment and to describe the production system.

Materials and methods

Study area

The study was carried out in Remada region (Tataouine district; Lat. 32°17.926'; Long. 10°34.818') in Southern Tunisia (Figure 1). The annual temperature averages are 9 and 29°C in January and August, respectively with a mean annual rainfall of 60 mm (Climate-Data.org).

The livestock population is characterized mainly by the production of camels and small ruminants. The production system is extensive mainly based on the use of communal rangelands. Livestock herds and flocks are characterized by a cyclic transhumant pattern between the eastern and the western boundaries. This is a very low input system with very little investment in animal buildings, feed supplementation and health care.

Figure 1: Geographic localisation of Remada region, Tunisia

Characterization of the studied environment

The current survey was carried out during 9 months between April 2018 and January 2019 to monitor the activity dynamics of *Hyalomma dromedarii*. In order to study the correlation between tick abundance and environmental factors, the humidity, temperature and the monthly rainfall were obtained from meteorological stations. The altitude and GPS coordinates were recorded with GPS device. Information related to the vegetation and soil type was also collected.

General view of the project target area

Animals and samples

A bimestrial survey of a randomly selected herd comprising between 30 and 70 Maghrebi camels maintained under extensive breeding system was conducted for tick infestation. The herd is mainly composed of female dromedaries. Herds are characterized by regular movements from East (Ouara) to West (Dhaher) in spring according to feed availability and from the West to East during the summer and autumn for watering. During this movement, camels can be in contact with herds originating from Libya.

Ticks were manually removed from camels and placed in flasks containing 70% ethanol. The specimens were morphologically identified at the laboratory using stereomicroscope according to the key of Walker et al. (2003). Ticks were then used for DNA extraction and DNA amplification by PCR. Information concerning the tick species and their attachment sites were recorded in the data sheet.

Ticks collection on camels

Blood samples were collected from jugular vein in EDTA and dry tubes, used for Giemsa staining, DNA extraction and sera collection. The objective is to determine the presence of *R. aeschlimannii* and to screen for CCHFV antibodies using ELISA tests.

DNA extraction, PCR and sequencing

In order to study the genetic diversity of *Hyalomma dromedarii* population, DNA was extracted from ticks with the Wizard® Genomic DNA purification kit (Promega, Madison, USA) following the manufacturer's instructions. DNA was stored at -20°C until used.

PCR will then be performed to amplify 16S rDNA and CO-1 genes of *H. dromedarii* using the 16Sr DNA and CO-1 primers.

PCR reaction will be performed in a final volume of 25 µl as described by (Kaur et al., 2016). PCR products will be analysed on a 2% agarose gel electrophoresis. A total of 100 PCR products will be purified and sequenced using the same primers.

Data analysis

All chromatograms for both 16S rDNA and CO1 of *Hyalomma dromedarii* will be visualised and edited manually to correct base calling errors and multiple sequence alignments will be performed using MEGA7 software and the sequences will be compared with the GenBank database with BLAST (Tamura et al., 2011). A phylogenetic tree will be constructed to explore the genetic diversity of *Hyalomma dromedarii* tick species and compare it with other tick strains in the world.

Tick-borne pathogens

In order to check the presence of *Rickettsia aeschlimannii* DNA in both ticks and camels, Catch- all primers (107F and Rm 299) recognizing genes encoding gltA and rOmpA will be used. Reactions will be performed in 25 µl volume following the programme developed by Ereqat et al. (2016) and Kamani et al. (2015). Positive samples will be tested for specific PCR reaction using specific primers R aesSca2 F, R aesSca2 R recognising 16Dr RNA gene (Djerbouh et al., 2012).

Enzyme-linked immunosorbent assay (ELISA)

Indirect enzyme-linked immunosorbent assay (ELISA) will be performed to screen the sera for CCHFV-specific IgG antibodies (Adam et al., 2013).

Statistical analyses

The results are expressed using two parasitological indicators (Bush et al., 1997):

Infestation prevalence (%) =100× (number of infested animals÷ number of examined animals); Tick infestation intensity= number of ticks ÷ number of infested animals

Preliminary results

Between April and November 2018, a total number of 719 ticks were collected. The population was dominated by *Hyalomma impeltatum* (N= 369) followed by *Hyalomma dromedarii* (N=208) and *Hyalomma excavatum* (N=142). The number of males was slightly higher than females (sex ratio M:F=1.02). The highest infestation prevalence was recorded in April (96%). The mean infestation intensity varied between 3 and 5 ticks/animal. The preferential attachment sites are anus (28%) and udder (13%).

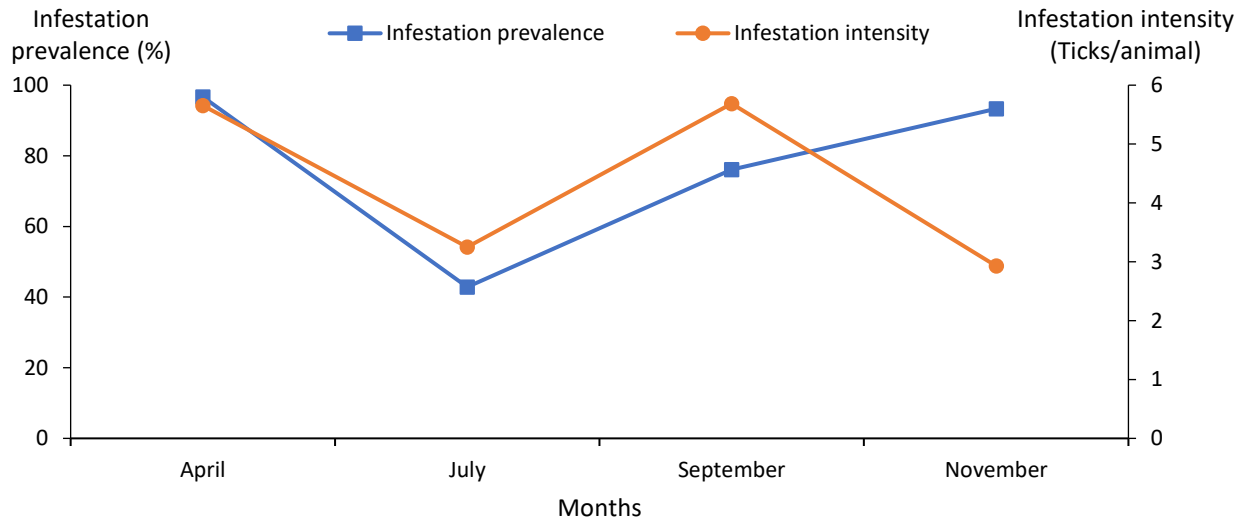

Figure 2: Monthly variation of infestation intensity and prevalence

On-going activities

Sampling and data collection will continue during 2019. The main expected outputs by the end of 2019 will be:

1. Characterisation of the environment of *Hyalomma dromedarii* and its host: description of biotope (vegetation and soil type), parameters related to ticks (tick density, infestation prevalence, attachment sites, oviposition, population distribution (radial distribution), description of the animal's history (origin, local and type of pastures,

nomadic or sedentary production type, across borders movements, map of the movement of camels during the year).

2. Study of the influence of presence of common water sources/pastures and possible infestation from other animals; possibility of presence of new tick species infesting camels.
3. Description of the activity dynamics of Hyalomma dromedarii in camels in southern Tunisia.
4. Phylogenetic analyses and genetic diversity of Tunisian Hyalomma dromedarii population.
5. Genetic characterization of pathogens Rickettsia spp., Theileria spp., transmitted by *Hyalomma dromedarii.*
6. Molecular detection of Rickettsia aeschlimannii from camel and ticks.
7. Serological detection of Crimean Congo Haemorrhagic fever antibodies in camels
8. Recommendations for a control programme against free-living instars and those present on camels.

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