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Progress report on modeling sheep ticks and tick-borne pathogens in Tunisia

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Contents

I State of the art and preliminary work

In Tunisia, sheep are exposed to multiple parasites and pathogens, which has a high economic impact and handicap market access, especially for poor farmers. *Rhipicephalus* spp. and *Hyalomma* spp. ticks are competent vectors of wide range of pathogens in sheep. Those pathogens have either economic impact by affecting sheep health, like *Babesia ovis* or have public health impact like *Anaplasma phagocytophilum*, Crimean Congo Hemorrhagic Fever virus…etc.

Studies on tick-borne pathogens (TBP) in sheep in Tunisia are scattered and need to be combined with a study of the biology of the tick vectors and environmental factors. Since, some TBP are zoonotic, well understanding the mechanism of interactions between ticks-host-environment is required to implement an efficient control program against ticks and TBPs.

Tunisia has a rich variety of bioclimatic area, ranging from the humid to the Saharan, in the northern and the southern country, respectively. The climate is Mediterranean in Northern part with rainy winters (1500 mm) and in South, the maximum rainfall doesn't reach 200 mm. Environmental variables are known to be effective contributors to habitat suitability of almost all tick species. Among those variables, annual precipitation (Bio12), mean temperature of the driest quarter (Bio9), minimum temperature of the coldest month (Bio6), and mean Normalized Difference Vegetation Index (NDVI) are involved in tick distribution and are usually considered in modeling it (Kessler et al., 2019). While the regional distributions of tick species were investigated by several studies, more localized distribution estimates has never been done in Tunisia and the effect of environmental factors on TBP has never been studied before.

We planed to build a model to predict habitat suitability for ovine piroplasms in order to raise awareness about sheep tick-borne pathogens in Tunisia.

II Study hypothesis

Temperature, precipitation and NDVI influence ticks and tick-borne pathogens distribution in Tunisia among the northern, the central and the southern parts of the country.

III Objectives

The objective of this work was to model sheep TBP distribution in Tunisia using environmental factors (temperature, precipitation and NDVI) and to give practical recommendations to decision makers according to the outputs.

IV Methodology

4.2. Study regions and protocol design

In Tunisia, there are 5 bioclimatic stages. To represent each bioclimatic stage, we selected the following areas (Figure 1):

- Humid: Fernana (Jendouba district)
- Sub-Humid: Mornaguia (Manouba district)
- Semi-arid: Saouaf (Zaghouan district)
- Arid sup (high steppes): Sebeitla (Kasserine district)

- Arid sup (low steppes): Bir Ali (Sfax district)
- Saharian: Bir Lahmar (Tataouine district)

Figure 1: Map of Tunisia (selected areas are indicated with red dots)

Two to three flocks of middle size and extensively managed were included from each region. GPS coordinates (latitude, longitude, altitude) were recorded. Adult ewes of different breeds were included in the sample and were ear tagged during the first visit. Then each trimester, all selected animals were sampled and present ticks were collected from infested animal ears. Seven rounds of visits were done between April 2018 and October 2019.

4.2. Ticks occurrence

Ticks collected from sheep were identified to species level under a stereomicroscope according to the key of Walker et al. (2003).

4.4. Tick-borne pathogens identification

The detection of pathogens was based on molecular tools (Polymerase chain reactions, PCRs) for *Babesia* and *Theileria* spp. The results were showed as Presence/Absence of pathogens. Presence of *Babesia*/*Theileria* corresponds to positive animal at least during one visit.

4.4. Environmental factors

The most relevant environmental variables affecting ticks distribution were identified from the literature. The main environmental factors that influence ticks and TBP distribution are temperature and precipitation hence NDVI may influence tick sheep distribution (Vajana et al., 2018) There are several sources of data providing temperature and precipitation variables: WorldClim database (v.1.4. release3) at a spatial resolution of 30 arc-seconds and in the un-projected latitude/longitude coordinate reference system (WGS84 datum). National Institute of Meteorology in Tunisia also provides daily values of those variables. NDVI will be deduced from eMODIS product (ex. Google earth engine).

4.4. Raster and vector data

Tunisia map at vector format was downloaded from www.diva-gis.org (WGS 84 datum). All raster files were transposed into Africa Albers Equal Area Conic projection to guarantee a constant pixel size and meet the main assumption of the statistical technique used to model TBP occurrence.

4.5. Species distribution models

R statistical software was used for developing the model. R packages MaxEnt was used to model *Babesia*/*Theileria* occurrence (ψx) from presence only data, by maximizing the probability of occurrence according to the logit-linear model as described by Vajana et al. (Vajana et al., 2018).

V Preliminary results

5.1. Ticks infesting sheep in Tunisia

Among all sampled animals and during the 7 visit rounds, the total number of collected ticks was 675. The most frequent tick species (91.7%) was *Rhipicephalus sanguineus* s.l. Few specimens of *Hyalomma impeltatum* (4.4%) and *Hy. excavatum* (2.22%) and less than 1% of *Hy. marginatum*, *Hy. dromedarii* and *Rh. bursa*, were also found (p<0.001)(Table 1).

Table 1: The total number of tick species collected from sheep during 7 successive trimesters sampling

	Number of tick species (%)							
Tick species	**April 2018**	**July 2018**	**October 2018**	**January 2019**	**April 2019**	**July 2019**	**October 2019**	**Overall**
Rhipicephalus sanguineus s.l.	144 (99.31)	60 (95.24)	17 (85)	5 (100)	149 (94.3)	184 (96.34)	60 (64.52)	619 (91.70)
Hyalomma impeltatum	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	3 (1.57)	26 (27.96)	30 (4.44)
Hyalomma excavatum	0 (0)	0 (0)	2 (10)	0 (0)	7 (4.43)	0 (0)	6 (6.45)	15 (2.22)
Hyalomma marginatum	0 (0)	1 (1.59)	0 (0)	0 (0)	0 (0)	3 (1.57)	1 (1.08)	5 (0.74)
Rhipicephalus bursa	1 (0.69)	2 (3.17)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.44)
Rhipicephalus annulatus	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.63)	1 (0.52)	0 (0)	2 (0.30)
Hyalomma dromedarii	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.63)	0 (0)	0 (0)	1 (0.15)
Overall	145 (21.48)	63 (9.33)	20 (2.96)	5 (0.74)	158 (23.41)	191 (28.30)	93 (13.78)	675 (100)

5.2. Molecular prevalence to piroplasms

The overall molecular prevalence of piroplasms during the total period was estimated to 1.36% (21/1544). The highest molecular prevalence was recorded in both July and October 2018 (13/36), while in April 2018 and January 2019, only 2/36 and 8/36 animals were infected. Among the 21 infected sheep, 9, 6, 4 and 2 were from Mornaguia, Fernana, Saouef and Tataouine, respectively (Table 2). All but one, infected sheep by piroplams carried also ticks at the moment of sampling.

Table 2: Molecular prevalence of *Theileria*/*Babesia* in sheep during four successive seasons rounds from April 2018 to January 2019

Animal ID	**Farms ID**	**Location**	**Latitude**	**Longitude**	**Piroplasms' positive PCR**
826	MOR2	Mornaguia	36,66071667	9,959313889	3
827	MOR2	Mornaguia	36,66071667	9,959313889	2
828	MOR3	Mornaguia	36,62245556	9,948786111	1
831	MOR3	Mornaguia	36,62245556	9,948786111	1
832	MOR3	Mornaguia	36,62245556	9,948786111	2
843	MOR3	Mornaguia	36,62245556	9,948786111	2
851	MOR3	Mornaguia	36,62245556	9,948786111	2
852	MOR3	Mornaguia	36,62245556	9,948786111	1
869	MOR3	Mornaguia	36,62245556	9,948786111	1
892	FER1	Fernana	36,70277778	8,807222222	1
1110	FER1	Fernana	36,70138889	8,807222222	1
1112	FER1	Fernana	36,70138889	8,807222222	1
1113	FER1	Fernana	36,70138889	8,807222222	1
1117	FER2	Fernana	36,70138889	8,807222222	3
1154	FER3	Fernana	36,70138889	8,807222222	1

1183	TAT3	Tataouine	33,12527778	10,565	2
1197	TAT3	Tataouine	33,12527778	10,565	1
997	SAO1	Saouaf	36,2575	10,14694444	3
1021	SAO2	Saouaf	36,24777778	10,15194444	1
1044	SAO3	Saouaf	36,22820556	10,17976944	2
1059	SAO3	Saouaf	36,22820556	10,17976944	4

5.3. Risk map for piroplasms occurrence and discussion

The suitability map obtained using MaxEnt model, showed that the occurrence of piroplasms in sheep, follows a North-South gradient. The most suitable region for ovine piroplasms is the Northern part of Tunisia, then few suitability foci in the South (marked by green color on the map) (Figure 2). The relative occurrence rate as shown in Figure 2 is only explained by temperature and humidity. Other factors such as NDVI and sheep density should be included in the model and may change the ROR prediction. Further studies are needed to go insights in this spatial analysis.

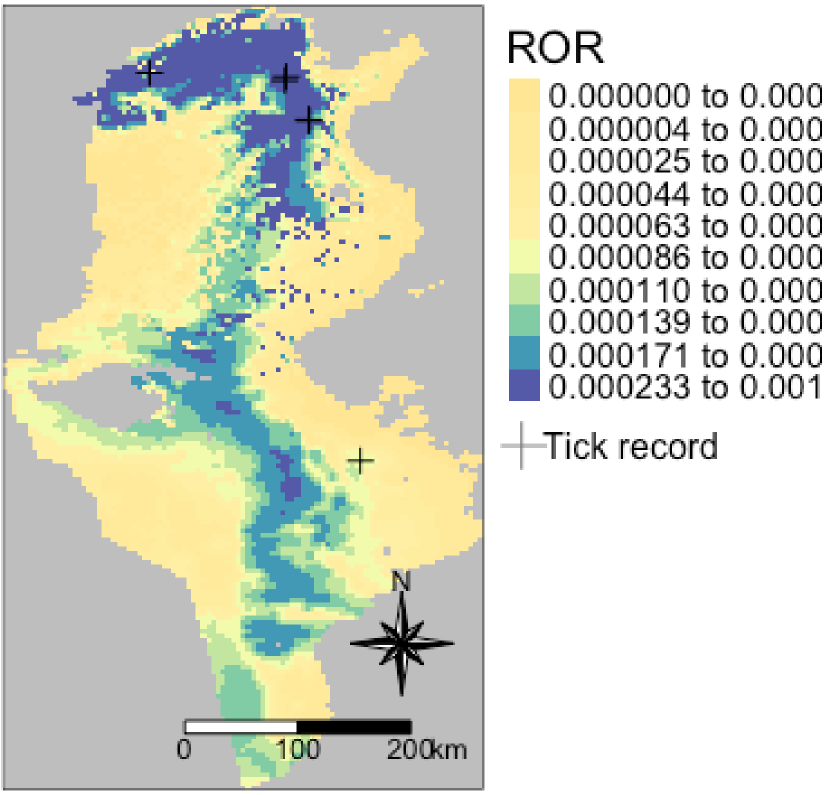

Figure 2: Predicted spatial distributions for *Babesia*/*Theileria* pathogens. ROR is the relative occurrence rate, showed in color from yellow to blue tones, which corresponds to increasing values of *Babesia*/*Theileria* occurrence. Sampled positive farms are represented with “plus” symbol.

VI Workplan for 2020

The data available from the present study, are not only presence data, but contains the number of ticks in each season, which should be incorporated to the risk map. Generalized linear model (GLM) could be applied to take into account the tick number in the model accordingly.
As the last field visit toke place in January 2020, it would be of paramount interest to add its results to this analysis.

Bioclimatic variables (BIO) collected from WroldClim, are referring to the period between 1960 and 1990, while tick occurrence in the present study were recorded from 2018 to 2020, we need to work with new bioclimatic data (national and free available data) and find the way to adjust them.
The risk map above was built taking into consideration only temperature and humidity. It would be very interesting if NDVI and animal density would be added to the model, as they are significant contributors to tick biology.

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