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

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ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

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Contents

I State of the art and preliminary work

Sheep are considered neglected in comparison to cattle. This attitude might be explained by: relative resistance to pathogens, simple nutrition needs and less complicated flock management. However, sheep are exposed to multiple parasites and pathogens, especially pathology of parasitic origin, which has a deeply economic impact and handicap market access. Studies on TBP in small ruminants in Tunisia, still scattered and need to be combined with a study of the vector ticks biology. Since, some TBP are zoonotic, well understanding the mechanism of interactions between ticks-host is required to implement an efficient control program against ticks and TBP. 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 winter and in South, the maximum rainfall doesn't reach 200mm. As the climate has a wide influence on the vegetation, the soil composition, it influences directly the vectors' species and distribution, and also sheep flocks' scattering along the country. The studies conducted until know were descriptive (listed in table 1) and abiotic factors (humidity, temperature, rainfall, soil composition…) were never been considered in tick borne pathogen distribution analysis. In another side, small ruminants play an important role in Tunisia, at economic and socio-cultural levels. Unfortunately small ruminant farmers face several natural and economic constraints: remote from the health care services, extreme climate conditions (very low temperature in winter in north and very hot in south), access difficulty to facilities (mountainous regions), low income for farmers, poor fodder resources and absence of breed genetic improvement. Indeed, in Tunisia two major breeds namely Barbarine with large tail sheep and Queue Fine de l'Ouest (Fine tail sheep) are the main meat breeds. Contrarily to cattle, the role of breed in tick infestation has never been studied in sheep. It might be of paramount importance to select resistant sheep breed to tick infestation and avoid misuse of acaricid medication that have a lot of drawbacks on human, animals and environment.

Table 1: Literature summary on sheep TBP in Tunisia

TBPathogen	Year	Region	Investig. tool	Prevalence (N)	Author
Coxiella	2005	Not mentionned	Not mentionned	7%-12%	(Rekiki *et al.*, 2005)
Coxiella	2008	Fahs (Zaghouan)	Serology (ELISA)	0.9%(N=224)	(Khamassi Khbou *et al.*, 2009)
Coxiella	2009	N.I.	qPCR	19%	(Berri *et al.*, 2009)
Theileria ovis	2013	Northern	RLB+PCR	28.1%(N=263)	(M'ghirbi *et al.*, 2013)
Anaplasma ovis	2014	Alia (Bizerte) Sbikha (Kair)	LAMP-Seq	70.2%(N=204)	(Belkahia, Ben and El, 2014)
Anaplasma ovis	2015	Bizerte Béja	PCR+Seq	93.8%(N=260)	(Said *et al.*, 2015)
Mycoplamsa ovis	2015	Kairouan Ariana	PCR+Seq	6.28%(N=573)	(Rjeibi *et al.*, 2015)
Borrelia s.l.	2016	Béja Bizerte	qPCR	6.2%(N=260)	(Said *et al.*, 2016)
Babesia ovis	2016	North Center South	PCR-Seq	7.8%(N=270)	(Rjeibi *et al.*, 2016)
Theileria ovis				16.3%(N=270)	
Crimean-Congo haemorrhagic fever virus	2016	North+South	RT-PCR	Human (5/181)	(Wasfi *et al.*, 2016)
A. phagocytophilum like 1 and 2	2017	Bizerte Béja Ariana Nabeul Tunis	PCR+RFLP	3.9%(N=355) (7% A. like 1) (5.4% A. like 2)	(Ben Said *et al.*, 2017)
A. ovis and A. bovis	2017	Bizerte Béja Ariana Nabeul Tunis	PCR+Seq	Spring 30.7%(n=355) Summer 36.1%(n=249) Fall 43.6% (n=236) Winter 31.8%(n=132)	(Belkahia *et al.*, 2017)

II Study hypothesis

- Sheep are exposed to several tick species in Tunisia under different agro-ecological and production systems. The role of these systems' transformation on tick infestation remains poorly understood.
- The tick-borne pathogens (TBP) available data and results could be used for modelling TBP distribution in sheep in order to predict their occurrence under specific

climate factors. The gap analysis resulted from this study could pave the way to further pathogens identification;

- Analyses of TBP regarding the biotic (breed, age, gender, herd management...) and abiotic factors (temperature, humidity, rainfall) were never been considered and their role in tick infestation, attraction and resistance never studied.

III Objectives

The give responses to the studied hypothesis, the following objectives were fixed:

1. Assessment of sheep farmers', veterinarians and DVM students perception regarding ticks and TBP;
2. Identification of ticks species infesting sheep in the study area and improve understanding of TBP regarding biotic and abiotic factors;
3. Modelling sheep TBP distribution in Tunisia;

IV Methodology

4.1. Perception surveys

The ticks and TBP perception were assessed using paper questionnaires for students and veterinarians surveys and using Open Data Kit collect (v1.18.2) application for farmers. The data were entered to Excel sheet and will be analysed using SPSS (version 21, IBM).

4.1. Ticks and tick-borne pathogens study

Protocol design

Herds with middle-sized animals were selected, they are applying extensive management and do not treat ticks. Adult ewes of Barbarine (Fat-tailed breed), Queue Fine Ouest (Thin-tailed breed), and cross-breeds were included in the sample and were identified by ear tags the first visit. Then each trimester, all selected animals were sampled as summarised in table 1. Two to three flocks of middle size were included from each region.

Study area

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

- Humid: Ain Draham (Jendouba district)
- Sub-Humid: Mornaguia (Manouba district)
- Semi-arid: Saouaf (Zaghouan district)
- Arid sup (high steppes): Sbitla (Kasserine district)
- Arid sup (low steppes): Bir Ali (Sfax district)
- Saharian: Tatouine (Tataouine district)

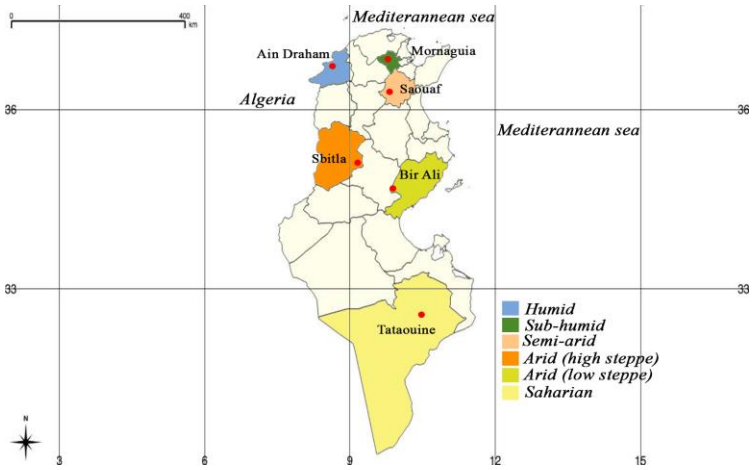

Figure 1: Map of Tunisia (dots in red are selected areas)

Flocks

Seventeen middle-sized flocks were randomly selected. The number of sampled animals in each region ranged between 59 and 90. The protocol design targeted only flocks with Barbarine and QFO breeds, but, it was not possible because of the unbalanced distribution of both breeds in the country.

Animals

Only adult females were selected in the sample, because they are immunized against tick infestation. Moreover, adult females are kept longer in the flocks, which enable annual monitoring of the tick infestation. In each selected flock, all adult females were ear-tagged and sampled. Each visit, data about general status (temperature measure, mucous inspection and body score measurement) was monitored. The data about herd management (feeding, watering, grazing…) were recorded the first visit. Blood, sera, ticks and feces were also collected (Table 3).

Table 2: Type of samples to collect for the study

Field samples collection	Volume/Quantity	Laboratory processing/ analysis
Total blood	5 ml	Hematological analysis Staining Giemsa DNA extraction (PCR)
Sera	5 ml	Antibodies against TBP
Feces	10g	Worms and eggs counting
Ticks	All	Identification Half preserved Half ➔ DNA extraction

V Progress

5.1. Perception surveys

Ticks and TBP perception surveys were conducted in three countries from North Africa: Algeria, Tunisia and Egypt (Table 1). The data entry of finalized surveys is undergoing on Excel sheet.

Table 3: Total of perception surveys about ticks and tick-borne pathogens in North African countries

	DVM Students	Veterinarians	Farmers
Algeria	166	46	Undergoing
Tunisia	118	85	89
Egypt	276	33	40
Totals	560	164	129

5.2. Total animals sampled each visit

A total of 1555 samples (blood, sera) were collected during the four visits in the six selected areas, 461, 389, 366 and 339 in April, July, October and January, respectively (Table 6). The number of animals decreased in all the regions from a visit to another, because some are dead or sold.

VI Preliminary results

6.1. Tick infestation indicators

The overall infestations' prevalences were 12.8%, 9.41%, 3.86% and 1.2% in April, July, October and January respectively ($p<0.001$). In April, the animals from Saouef locality were significantly most infested than other from Fernana, Mornaguia and Sbitla regions ($p<0.001$). Whereas in July, Fernana was most infested area than Saouaf, Mornaguia, Bir Ali and Mornaguia ($p<0.001$). Tataouine, from the Southern Tunisia, was most infested than the other regions together, in both October and January ($p<0.001$)(Table 6).

Table 4: Total animals infested according to the season and the area studied

	April 2018	July 2018	October 2018	January 2019
	Infested/examined			
Mornaguia	7/90	6/60	2/61	0/61
Fernana	12/75	20/65	0/63	0/56

Tataouine	0/59	0/44	12/40	4/34
Sbitla	3/89	1/88	1/78	0/73
Bir Ali	0/66	6/57	3/58	0/54
Saouaf	37/82	13/74	0/66	0/60
Totals	59/461	45/389	18/366	4/339

The number of collected ticks, were about 146, 63, 20 and 6 in April, July, October and January respectively ($p<0.001$). The ticks infestation intensity and tick abundance were inversely proportional to temperature, which is colder in the fall (October) and in winter (January) (Table 7; Figure 2).

Table 5: Tick infestation prevalence and intensity and tick abundance according to season

	April 2018	July 2018	October 2018	January 2019	p
Infestation prevalence	0.13	0.09	0.04	0.01	0.001
Infestation intensity	2.47	1.37	1.11	1.50	0.003
Abundance	0.31	0.13	0.04	0.01	<0.001

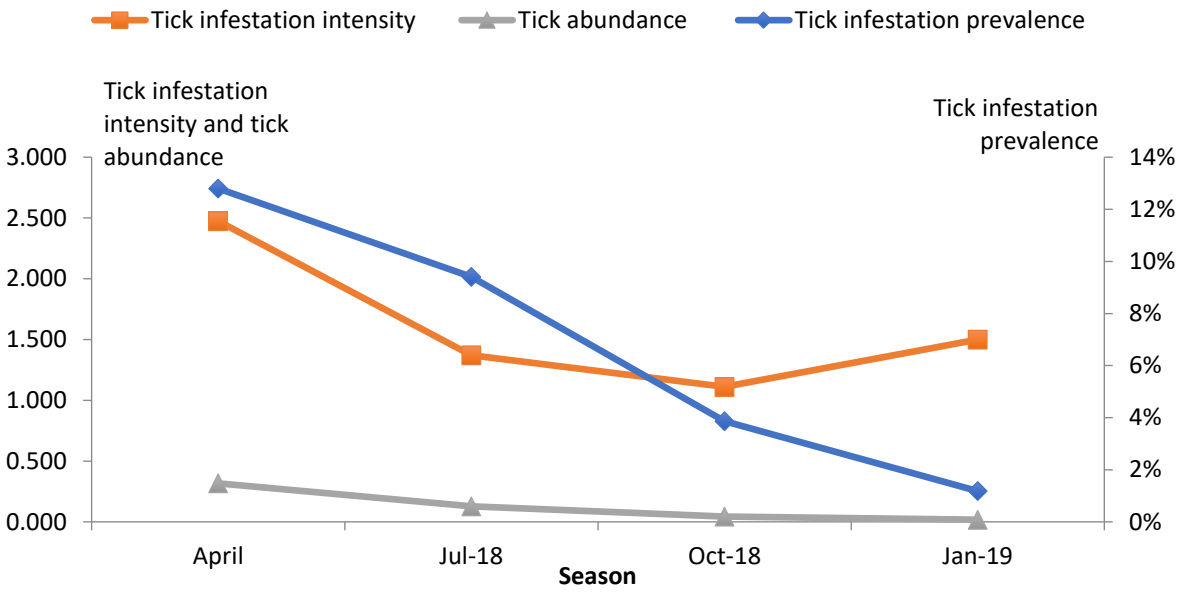

Figure 2: Ticks' infestation prevalence and intensity and ticks abundance according to season

6.2. Tick-borne pathogens identified by Giemsa staining

The Giemsa staining allowed the identification of forms that might be associated either to *Anaplasma* spp., or *Babesia* spp. or *Theileria* spp. parasites. The *Anaplasma* spp. was the most viewed forms on the examined slides (Table 6).

Table 6: Piroplasms and *Anaplasma* forms identified by Giemsa staining

	April 2018	July 2018	October 2018
Anaplasma	133	34	64
Babesia	16	3	3
Theileria	4	2	1
Totals	153	39	68

VII Conclusion

The preliminary descriptive results showed that, tick infestation among sheep population in Tunisia is variable regarding season and region. In April and July, tick infestation was higher in humid and semi-arid regions (Fernana, Mornaguia and Saouaf). In October and January, the saharian region of Tataouine in Southern Tunisia was the most affected area. As next step, it will be interesting to identify the tick species involved in tick infestation and tick-borne pathogens transmitted. Also it will be of paramount importance to analyse all these data regarding the herd management conditions, the sanitary situation of herds and the climatic factors. Are undergoing in the laboratory now the identification of ticks according to Walker et al., 2014, at both genus and species levels, also the identification of digestive parasites (worms) and the DNA extraction from blood.

VIII Publications, conferences and trainings

8.1. Publications and communications

- Elati K., Ayadi A. A., **Khamassi Khbou M.**, Gharbi M., Population dynamics of ticks infesting sheep in the arid steppes of Tunisia, published in the “Revue de l’élevage et de medicine vétérinaire des pays tropicaux, in October 2018.
- Elati K., Chiha H., **Khamassi Khbou M.**, Gharbi M., Activity dynamics of *Gasterophilus* spp. Infesting donkeys (*Equus asinus*) in Tunisia, published in “Advances in parasitology” in May 2018. (DOI: 10.17582/journal.jap/2018/5.1.1.5)
- “Using ODK in animal health surveys” in the 2nd Seminar of the National School of Veterinary Medicine of Sidi Thabet, Tunisia, the 26th October 2018.
- “The infectious causes of anaemia in ruminants”; the Post-Graduate Course about “Anaemia in Ruminants”, held in the NSVM, Tunisia, the 31th October 2018.
- “The values of complementary tests in canine practice” the Post-Graduate Course about “Complementary tests in canine practice”, held in Friguia Parc, Ennfidha, Tunisia, the 3rd November 2018.
- “Using ODK in animal health surveys” in the 9th Seminar of The Institute of veterinary sciences of Constantina, Algeria, the 16th December 2018.

8.2. Trainings

8.2.1. Médiha Khbou Khamassi

1. Workshop on Google Earth Engine, National School of Veterinary Medicine of Sidi Thabet, Tunisia, 28th and 29th March 2018;

2. 6th military health course in medical writing methodology, Ras Tabia, Tunisia, from 2nd to 6th April 2018.
3. Training on how using Open data kit, ICARDA, Cairo, Egypt from 4th to 6th June 2018.
4. “Doctoriales” of Manouba University, Tunisia, from 26th to 30th November 2018.
5. “Using SPSS” provided by the Training Engineering Center, in Menzah 1, from the 12th to the 13th January 2018.
6. “Bases in GIS”, provided by the Training Engineering Center, in Menzah 1, from the 2nd to the 3rd February 2018.

8.2.2. Rihab Romdhane

1. «Actualités en pathologie digestive chez les équidés» 18th & 19th January 2018: Post-University course at the National School of Veterinary Medicine of Sidi Thabet, Tunisia.
2. Participation to the scientific seminar of the Order National Council of Veterinary Medicine Doctors, Nabeul, Tunisia, 27th & 28th January 2018.
3. Participation to seminiar about «Permaculture», at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, 5th & 6th February 2018.
4. Participation to the workshop «Isolement des parasites de la viande », at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, 21st February 2018.
5. Participation to the workshop « Introduction to the geographic Information System in animal health » organised by the ICARDA at the National School of Veterinary Medicine of Sidi Thabet, Tunisia 28th & 29th March 2018.
6. Participation to the training «How to make bibliographic research in CABI» organised by the CNUDST at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, 11th April 2018.
7. Participation to the scientific congress of the Order National Council of Veterinary Medicine Doctors, 20th and 21th April 2018.
8. Participation to the workshop « *Theileria annulata* vaccination, lessons from history » at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, 25th and 26th June 2018.
9. Participation in the workshop « Using Open Data Kit in animal health surveys », at the National School of Veterinary Medicine of Sidi Thabet, Tunisia, 26th and 27th July 2018.
10. Participation at the training « Good clinical Practice/ Good Clinical Laboratory » at the Pasteur Institute of Tunis, Tunisia, 4th to 6th September 2018.
11. Participation at the training « Ethique de l’expérimentation animale » at the Pasteur Institute of Tunis, Tunisia, 27th September 2018.
12. Participation to the Post-Graduate Course « Les anémies chez les ruminants » at the National School of Veterinary Medicine of Sidi Thabet, Tunisia 31th October and the 1st November 2018.

8.3. Teaching activities

1. 1st summer school of statistics in animal health 21th, 22th and 23th June 2018 in the NSVM, Tunisia.
2. Using ODK in animal health surveys, 26th, 27th, 30th and 31th July 2018 in the NSVM, Tunisia.
3. Post-University course “Anaemia in Ruminants”, 31th October and 1st November 2018 in the NSVM, Tunisia.

4. Post-University course on “Complementary tests in canine practice”, Friguia Ennfidha, 3rd November 2018.

8.4. Travelling

1- Egypt:
   a. From the 4th to the 6th June 2018 in Cairo for training on using ODK with Mr Khaled Al-Shamaa and getting three tablets Samsung.
   b. From 6th to 12th June 2018 in Assiut to make ticks and TBP perception survey with farmers from Assiut district.
2- Algeria (Batna): from 4th to 7th to participate to the Seminar of Algerian Veterinarians Practitioner and collect data about their perception on ticks and TBP.
3- Algeria (Constantina), from 15th to 17th December 2018 to participate to the 9th seminar of the Veterinary Sciences Institute of Constantina

8.5. Capacity building

Farmers that are involved in the study have received anthelmintic drugs, vitamin mineral supplement foods and acaricid and boots.

IX Workplan for 2019

The year 2019 will be devoted to the achievement of:

1- Field visit for the second year and samples collection organization.
2- Preparation of reports to farmers with main results of laboratory analyses.
3- Laboratory analyses, mainly:
   2.1. DNA extraction from blood
   2.2. Molecular identification of tick-borne pathogens
   2.3. Serology for the detection of antibodies against tick-borne encephalitis virus, Louping ill virus and Crimean Congo Haemorraghic Fever Virus.
   2.4. Ticks identification
4- Data entry and analysis (prevalences, intensity of infestation, risk factors for TBP, perception regarding ticks and TBP, modelling of TBP…)
5- Publication of two articles: one about students, farmers and veterinarians ticks and TBP perception on ticks and TBP and another about TBP, in peer-reviewed international journals and participation in national and international scientific events.

Workplan for 2019