DOI: 10.1002/vms3.310

ORIGINAL ARTICLE

WILEY

Individual variability among autochthonous sheep in Northern Tunisia to infection by abomasum nematodes and *Babesia/Theileria* parasites

Mariem Rouatbi[1] | Rihab Romdhane[1] | Faten Bouaicha[1] | Rahma Saddem[1] | Limam Sassi[1] | Mokhtar Dhibi[1] | Mourad Rekik[2] | Aynalem Haile[3] | Joram M. Mwacharo[3] | Barbara Rischkowsky[3] | Mohamed Aziz Darghouth[1] | Mohamed Gharbi[1]

[1]Laboratoire de Parasitologie, Université de la Manouba, Sidi Thabet, Tunisia

[2]International Center for Agricultural Research in the Dry Areas (ICARDA), Amman, Jordan

[3]International Center for Agricultural Research in the Dry Areas (ICARDA), Addis Ababa, Ethiopia

Correspondence
Mariem Rouatbi, Laboratoire de Parasitologie, Université de la Manouba, École Nationale de Médecine Vétérinaire de Sidi Thabet, Sidi Thabet 2020, Tunisia.
Email: rouatbi.myriam@yahoo.fr

Funding information
Laboratoire d'Épidémiologie des Infections Enzootiques des Herbivores en Tunisie: Application à la Lutte (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, Tunisia), Grant/Award Number: LR16AGR01; CGIAR Research Program on Livestock (CRP Livestock)

Abstract

In Tunisia, livestock plays an important role in the economy; unfortunately, Tunisian sheep population faces several health challenges. The aim of this trial was to study phenotypic variability of four local sheep breeds and strains to abomasum nematodes infection and to *Babesia/Theileria* parasites. Faeces, blood and abomasum contents were collected from 310 sheep slaughtered in eight commercial slaughterhouses across North Tunisia. Haematological and biochemical parameters were assessed. DNA was extracted and catch-all primers were used to detect both *Theileria* spp. and *Babesia* spp. DNA. Faecal egg counts (FEC) was quantitatively assessed using simple flotation technique followed by McMaster technique. Male and female worms were collected from all abomasum contents and counted under a stereomicroscope. The percentage of faeces samples positive for GIN's eggs was 30.82%. After worms' recovery, the infection prevalence was estimated to 75.90%. The overall infection prevalence by *Babesia* spp. and *Theileria* spp. was 4.21%. The dispersion of observations plots obtained by principal component analysis (PCA) showed two clusters of individuals. The first cluster contains animals having positive *Babesia/Theileria* PCR, presence of nematodes in the abomasum contents and relatively low total worm count (TWC < 500) expect one animal which was found bearing high TWC (>500). In this same group, with a suspected form of resistance, animals showed normal values of albumin and normal haematological parameters (red blood cell count [RBC], haemoglobin [Hb] and packed cell volume [PCV]). The second cluster represents all the other observations in which subgroups of animals were distinguished on the basis of their potential resistance to abomasum nematodes. Multiple correlations showed significant positive correlations between RBC/Hb, RBC/PCV, PCV/Hb and FEC/TWC. Significant negative correlations were observed between TWC/RBC and TWC/Hb. It is concluded that the phenotypic variability among local sheep breeds is essential for more advanced genetic and genomic studies.

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.310

KEYWORDS
abomasum nematodes, breeds, phenotypes, sheep, Tunisia

1 | INTRODUCTION

In Tunisia, livestock plays an important role in the economy in terms of food security, employment and local production of animal source food. Sheep farming plays a vital role in graduating the poor rural communities from poverty and has a high social and financial importance (Rekik, Aloulou, & Ben Hamouda, 2005). In 2017, the agricultural sector contributed to 10% of the Gross domestic products (GDP) and recorded an average growth of 2.8% per year over the period 2011–2017, which was by far higher than figures of the national GDP (Chebbi, 2019). With a total population exceeding four million female units, this sector contributes to 48% of the total red meat production (Mohamed-Brahmi, Khaldi, & Khaldi, 2010). In Tunisia, there are four sheep breeds occupying distinct ecosystems from the north to the Sahara in the south (Rekik et al., 2005). However, the northern area which stretches over 24,000 km^2, characterized by mountainous and forest grazing areas, is different in a way that the four breeds coexist leading to the presence of various cross-bred animals which could reflect uncontrolled breeding practices by local farmers. Because of its topography, the north of the country seems to be the less-suited ecosystem for the country dominant fat-tailed Barbarine breed (Rekik et al., 2005). Tunisian sheep population is facing several health challenges including parasitic infections such as toxoplasmosis (Gharbi et al., 2013), fasciolosis (Akkari, Gharbi, & Darghouth, 2011), lungworms and gastrointestinal helminths (Akkari, Gharbi, & Darghouth, 2012) that cause persistent production and economic losses (Waller, 1997a). These losses are due to decreases in weight and milk yield as well as wool and mortality increase (Soulsby, 1983) and huge control costs (Akkari et al., 2014). In return, resistant parasite populations to several chemical anthelmintics are increasing (Jabbar et al., 2006). Due to the high cost of chemical anthelmintics, veterinary costs and sometimes their non-availability, small farmers are attempting to adapt ethno-veterinary practices as an alternative to control these parasites in several developing countries (Al-Shaibani, Phulan, & Shiekh, 2009; Deeba, Muhammad, Iqbal, & Hussain, 2009; Hussain, Khan, Iqbal, & Sajid, 2008; Sindhu, Iqbal, Khan, Jonsson, & Siddique, 2010). As gastrointestinal parasite resistance is under genetic control (Bishop, 2012; Bishop & Stear, 2003), genetic improvement could offer a solution to solve the problem caused by one of the most pathogenic gastro-intestinal sheep parasites: *Haemonchus contortus* (Kaplan, 2009).

Prior to genetic studies, phenotypic measurements are the first step to identify and characterize resistant animals. In Tunisia, the phenotypic and genetic resistance of the autochthonous sheep breeds to abomasum nematodes associated to *Babesia*/*Theileria* parasites, which represent an important animal health problem, have never been explored. The present work aimed at studying phenotypic variability of the local sheep breeds and strains to infection by abomasum nematodes and *Babesia*/*Theileria* parasites in the northern area of the country where animals extensively graze natural vegetation throughout the year, hence increasing their exposure to GIN infection (Waller, 1997b). To gain a better understanding of resistance to abomasum nematodes, phenotypic measurements were used, and different sheep clusters were identified.

2 | MATERIALS AND METHODS

2.1 | Study area and animals

The samples were collected from 310 animals slaughtered in eight commercial slaughterhouses across North Tunisia located in five governorates: Tunis (slaughterhouse of Tunis), Ariana (slaughterhouses of Ariana), Bizerte (slaughterhouses of Bizerte, Mateur and Sajnène), Béja (slaughterhouse of Béja) and Jendouba (slaughterhouses of Jendouba and Tabarka) (Figure 1, Table 1). Fieldwork conditions allowed that out of a total number of 310 animals, abomasum contents, faeces and blood were collected from only 307, 292 and 309 animals respectively. The samples were collected between September and December 2017; information about the sex, the breed and the age of the animals were collected before animal slaughtering (Table 2). Other general information was also collected from the owner, in particular the grazing pattern of the animals and the non-use of anthelmintic drugs during at least the year before.

2.2 | Sample collection and preparation

2.2.1 | Haematological and biochemical analyses

Blood samples were collected from each sheep by puncture of the jugular vein using sterile EDTA and heparinized tubes. EDTA tubes were used for DNA extractions and for haematological parameters estimation, namely red blood cell count (RBC) ($\times 10^6$/ml), haemoglobin (Hb) (g/dl) and packed cell volume (PCV) (%). Haematological measurements were performed with an Auto Haematology analyser BC-2800Vet® (Shenzen Mindray Bio-Medical Electronics Co., Ltd).

Plasma samples were collected from heparinized tubes and were used to estimate albuminaemia (ALB) (g/L) and total proteins (g/L) with a Random Access Clinical Autolyzer® (Dialab). Threshold values considered for haematological and biochemical parameters are shown in Table 3.

2.2.2 | Faecal egg counts

For all faeces samples, faecal egg counts (FEC) were estimated using 5 g of stool collected from each sheep by simple flotation technique

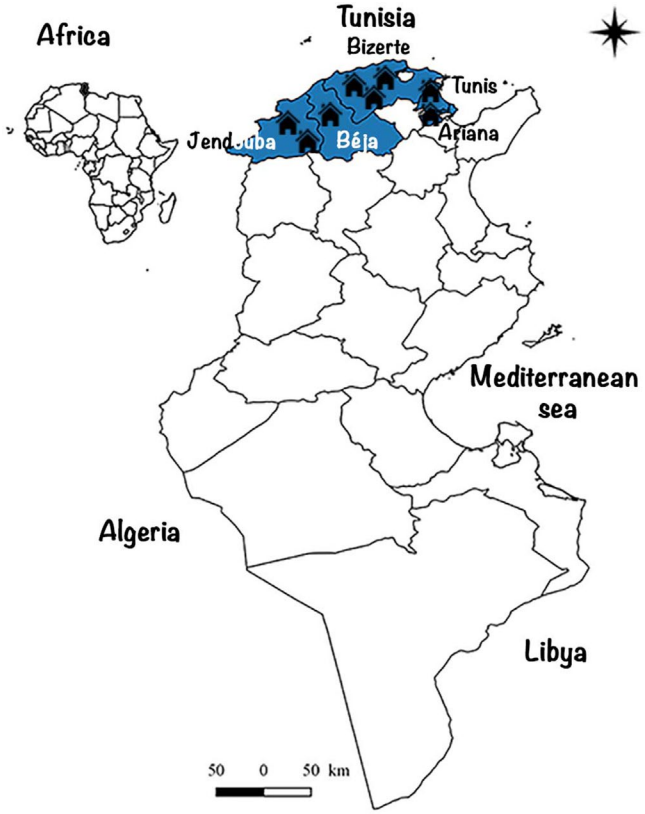

FIGURE 1 Districts delineating the sampling area in North Tunisia

followed by McMaster technique. Eggs of gastro-intestinal helminths (including nematodes and cestodes) and *Eimeria* spp. were identified. Eggs of *Trichuris* spp. and *Nematodirus* spp. were identified based on their specific morphology. For positive faecal samples, the quantification was done with the McMaster technique (Raynaud, 1970).

2.2.3 | Abomasum collection and worm recovery

As soon as the sheep is slaughtered and directly after removal of the intestinal tract, each abomasum was legated at both ends then cut and transported to the Laboratory of Parasitology at the National Veterinary School.

Each abomasum was opened along its greater curvature and its content was washed into a bucket. All abomasum nematodes were collected and conserved in ethanol 70% in identified Eppendorf tubes. As the identification of females is very difficult, they have been excluded and only abomasum male nematodes were identified according to the key of Euzeby (1982) and Taylor, Coop, and Wall. (2007).

2.2.4 | PCR amplification of *Theileria/Babesia* 18S rRNA gene

DNA was extracted from 300 µl of EDTA blood sheep samples using Wizard® Genomic DNA purification kit (Promega) according to the manufacturer's instructions then stored at −20°C until used. Catch-all primers (RLB-F and RLB-R) which detect both *Theileria* spp. and *Babesia* spp. piroplasms were used (Gubbels et al., 1999). These primers amplify the hypervariable V4 region of the 18S ribosomal ribonucleic acid (rRNA) gene of all *Theileria* spp. and *Babesia* spp. species. PCR reactions were performed in 25 µl volume consisting of 19 µl PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each deoxyribonucleotide triphosphate, 0.125 µg of Taq hot start Ab, 0.1 U of Uracil DNA glycosylase, 25 pmol of each primer and 1.25 U of Super Taq DNA polymerase (Vivantis) and 3 µl of DNA sample (Gubbels et al., 1999). PCR products were examined by electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

2.3 | Statistical analyses

Measurements related to haematological and biochemical parameters, FEC, total worm count (TWC) and *Babesia/Theileria* infection were collected and compiled in a single Excel database. Risk factors (sex, breed, location and age group) were analysed with the chi square of Mantel–Haenszel using Epi Info 6 (Dean et al., 2011). Parasitological indicators were calculated by estimating the percentage of sheep having positive DNA of *Babesia/Theileria*, positive faeces for GIN's eggs and presence of nematodes in the abomasum contents.

For GIN's eggs and abomasum nematodes the following parasitological indicators were estimated (Bush, Lafferty, Lotz, & Shostak, 1997):

Prevalence = 100 × number of infected sheep/number of examined sheep

Intensity = Number of collected worm in the abomasum/number of infected sheep

Abundance = Number of collected worm in the abomasum/number of examined sheep

Total Worm Count (TWC) = number of nematodes in examined abomasum

FEC = Number of GIN's eggs/gram of faeces

All parasitological indicators were presented as % (± standard deviation).

To describe the association between the infection status of the studied sheep (*Babesia/Theileria* and abomasum nematodes) and both their haematological and biochemical profiles, principal component analyses (PCA) were performed using SPSS® for Window® (IBM SPSS Statistics version 23, USA, 2010). For PCA, 6 variables were considered: *Babesia/Theileria* infection, presence or absence of nematodes in the abomasum contents, albuminaemia, RBC, Hb and

TABLE 1 Characteristics of the studied areas[a,b,c]

Location	District	Localization	Mean annual rainfall (mm)	Mean annual temperature (°C)	Mean temperature during winter (°C)	Mean temperature during summer (°C)	GPS coordinates	Total number of sheep heads (2015–2016)
Ariana	Ariana	North-east	449	18.0	11.3	24.9	36°51′N 10°11′E	32,400
Beja	Beja	North-west	662	17.7	9.9	26.1	36°44′N 09°11′E	357,560
Bizerte	Bizerte	North	527	18	11.6	24.5	37°16′N 9°52′E	199,670
Mateur	Bizerte	North	539	18	11.2	25.1	37°02′N 9°39′E	199,670
Sajnène	Bizerte	North	527	18	11.2	25.1	37°3′N 9°14′E	199,670
Jendouba	Jendouba	North-west	504	18	10.4	26.6	36°29′N 08°47′E	232,550
Tabarka	Jendouba	North-west	865	18.2	11.8	24.8	36°57′N 8°45′E	232,550
Tunis	Tunis	North-east	448	18.1	11.4	25.2	36°48′N 10°10′E	18,880

[a]Climatedata.org, 2018, URL: http://fr.climate-data.org/.
[b]Date and time.info, 2018, URL: http://dateandtime.info/.
[c]Ministry of Agriculture, Water Resources and Fishing, Tunisia (2016). Official statistics of the Tunisian Ministry of Agriculture, Survey on agricultural season (Livestock), general administration of studies and agricultural development.

TABLE 2 Characteristics of the sampled animals

Parameter	Number of animals (%)
Sex	
Male	90 (29.03)
Female	220 (70.97)
Breed	
Barbarine	37 (11.94)
Cross breed	142 (45.81)
Noire de Thibar	59 (19.03)
Queue Fine de l'Ouest	72 (23.23)
Location (district)	
Ariana (Ariana)	34 (10.97)
Beja (Beja)	54 (17.42)
Bizerte (Bizerte)	29 (9.35)
Sajnène (Bizerte)	40 (12.90)
Mateur (Bizerte)	50 (16.13)
Jendouba (Jendouba)	49 (15.81)
Tabarka (Jendouba)	25 (8.06)
Tunis (Tunis)	29 (9.35)
Age group (years)	
<1	243 (78.39)
≥1	67 (21.61)
Overall	310

TABLE 3 Sheep haematological and biochemical threshold normal values[a]

Parameter	Threshold value
Haematological parameters	
Red blood cells (RBC) (10^{12}/L)	5.0–14.0
Packed cell volume (PCV) (%)	26.0–45.0
Haemoglobin (Hb) (g/dL)	9.0–15.5
Biochemical parameters	
Albumin (g/L)	24–30
Total proteins (g/L)	60–79

[a]Blood and Radostitis, 1989.

PCV. Outliers, represented by scattered points that don't belong to any of the clusters, were not considered.

Infection prevalences and clusters' distribution were compared with the chi square of Mantel–Haenszel using Epi Info 6 (Dean et al., 2011). The concordance between simple flotation technique and worm recovery was estimated with the Kappa test (Toma, Dufour, Sanaa, & Bénet, 2007). The classification of Landis and Koch (1977) for the interpretation of the Kappa values was used: poor (<0); slight (0.01–0.20); fair (0.21–0.40); moderate (0.41–0.60), substantial (0.61–0.80), almost perfect (0.81–1.00). Multiple correlations tests were performed with SPSS® for Window® (IBM SPSS

Statistics version 23, USA, 2010) to assess correlations between haematological parameters (RBC, Hb and PCV), faecal egg counts (FECs) and total worm counts (TWCs). All performed statistical tests were considered significant at 5% threshold value (Schwartz, 1993).

3 | RESULTS

3.1 | Parasitological indicators

Among the 292 examined sheep faecal samples, 90 were positive for GIN by simple flotation technique giving an overall infection prevalence of 30.82% (±2.70). The number of sheep aged 12 months or more with positive faeces for GIN's eggs was significantly higher than for those aged less than 12 months [49.23 (±6.20) and 25.55% (±2.89) respectively; p = .001]. Animals belonging to the Barbarine breed had higher infection prevalence by simple flotation technique [55.56% (±8.28)] (p = .047) compared with Cross breed, Noire de Thibar and Queue Fine de l'Ouest sheep. Infection prevalence of animals from Béja [56% (±7.02)] and from Tabarka's slaughterhouses [58.33% (±10.06)] was higher than in sheep from the other 6 slaughterhouses (p < .001). There was no significant difference of infection prevalence by simple flotation technique according to sex (Table 4).

Among the 310 sampled sheep, 307 abomasum contents were collected of which 233 were infected by at least one nematode, corresponding to an overall infection prevalence of 75.90% (±2.44). As for faeces analyses, significantly higher infection prevalences were recorded in Barbarine breed, in the two slaughterhouses of Béja (Béja district) and Tabarka (Jendouba district) and for sheep older than 12 months (Table 4).

Eight nematode species were found in the abomasum: *Teladorsagia circumcincta* [55.16 (±2.82)], *Teladorsagia trifurcate* [34.87 (±2.82)], *Haemonchus contortus* [24.84 (±2.45)], *Marshallagia marshalli* [12.58 (±1.88)], *Trichostrongylus vitrines* [1.61 (±0.72)], *Trichostrongylus axei* [1.29 (±0.64)], *Ostertagia lyrate* [0.97 (±0.56)], *Ostertagia ostertagi* [0.65 (±0.45)] and *Ostertagia occidentalis* [0.65± (0.45)]. The overall mean infection intensity and abundance were estimated at 199.49 and 149.94 respectively. The highest worm mean infection intensity was recorded in a sheep from Bizerte district (n = 2,991). The kappa concordance coefficient between simple flotation technique and worm recovery was 0.21 which is a fair

TABLE 4 Association between *Babesia* spp./*Theileria* spp. infection, faeces analyses for GIN and abomasum infected with worms in sheep and different parameters

	***Babesia* spp./*Theileria* spp. PCR**		**GIN's eggs in faeces**		**Presence of nematodes in the abomasum contents**	
Parameter	**Positive/examined (%)**	***p* value**	**Positive/examined (%)**	***p* value**	**Positive/examined (%)**	***p* value**
Sex						
Male	2/90 (2.22 ± 1.55)	.26	21/85 (24.71 ± 4.68)	.14	68/90 (75.56 ± 4.53)	.9
Female	11/219 (5.02 ± 1.48)		69/207 (33.33 ± 3.28)		165/217 (76.04 ± 2.90)	
Breed						
Barbarine	1/37 (2.70 ± 2.67)	.95	20/36 (55.56 ± 8.28)	.047*	32/37 (86.49 ± 5.62)	.03*
Cross breed	6/142 (4.23 ± 1.69)		34/137 (24.82 ± 3.69)		112/139 (80.58 ± 3.36)	
Noire de Thibar	3/59 (5.08 ± 2.86)		20/58 (34.48 ± 6.24)		41/59 (69.49 ± 5.99)	
Queue Fine de l'Ouest	3/71 (4.23 ± 2.39)		16/61 (26.23 ± 5.63)		48/72 (66.67 ± 5.56)	
Location (district)						
Ariana (Ariana)	0/33 (0)	.64	6/29 (20.69 ± 7.52)	<.001**	23/34 (67.65 ± 8.02)	.002*
Beja (Beja)	3/54 (5.56 ± 3.12)		28/50 (56.00 ± 7.02)		48/52 (92.31 ± 3.70)	
Bizerte (Bizerte)	3/29 (10.34 ± 5.66)		10/29 (34.48 ± 8.83)		24/29 (82.76 ± 7.01)	
Mateur (Bizerte)	2/50 (4.00 ± 2.77)		6/49 (12.24 ± 4.68)		35/50 (70 ± 6.48)	
Sajnène (Bizerte)	2/40 (5.00 ± 3.45)		10/39 (25.64 ± 6.99)		25/39 (64.10 ± 7.68)	
Jendouba (Jendouba)	1/49 (2.04 ± 2.02)		9/46 (19.57 ± 5.85)		36/49 (73.47 ± 6.31)	
Tabarka (Jendouba)	1/25 (4.00 ± 3.92)		14/24 (58.33 ± 10.06)		24/25 (96 ± 3.92)	
Tunis (Tunis)	1/29 (3.45 ± 3.39)		7/26 (26.92 ± 8.70)		18/29 (62.07 ± 9.01)	
Age group (years)						
<1	10/242 (4.13 ± 1.28)	.9	58/227 (25.55 ± 2.89)	.001**	175/240 (72.92 ± 2.87)	**.02***
≥1	3/67 (4.48 ± 2.53)		32/65 (49.23 ± 6.20)		58/67 (86.57 ± 4.17)	
Overall	13/309 (4.21 ± 1.14)		90/292 (30.82 ± 2.70)		233/307 (75.90 ± 2.44)	

Abbreviations: GIN, gastrointestinal nematodes; p, probability.

*Significant (.001 ≤ p ≤ .05);

**Highly significant (p < .001).

agreement between the two techniques according to the interpretation of Cohen's Kappa values.

The overall infection prevalence in sheep by *Babesia* spp. and *Theileria* spp. was 4.21% (±1.14) (13/309); it did not significantly differ according to sex, breed, age and location ($p > .05$) (Table 4).

3.2 | Principal component analysis (PCA) results

Two components were retained and were sufficient to explain the variance. The component plot showed that haematological parameters (RBC, Hb and PCV) were positively correlated with component 1 (*x*-axis). *Babesia*/*Theileria* infection, presence/absence of nematodes in the abomasum contents and albuminemia were positively correlated with component 2 (*y*-axis) (Figure 2).

Each animal in the sample was plotted on the bases of its first and second component values. The dispersion of observations plots for PCA showed 2 clusters of animals; three animals were considered as outliers (Figure 3). The first one (red circle) represents animals having positive *Babesia*/*Theileria* PCR and relatively low TWC (0 < TWC < 500) expect one animal which was found with high TWC (>500). These animals had biologically normal values for both albumin and haematological parameters (RBC, Hb and PCV). The second cluster (green circle) represents all other observations with 98% of animals having negative DNA for *Babesia* and *Theileria*, 75% of animals' abomasum contents positive for nematodes and with variable haematological and biochemical parameters. Relationship between haematological and biochemical results in the two animal clusters is summarised in Table 5. Other phenotypic traits (age, sex, breed and location) are depicted in Table 6. In cluster 1, represented by the most resistant animals at the phenotype level, percentage of females belonging to the Cross breed was significantly higher.

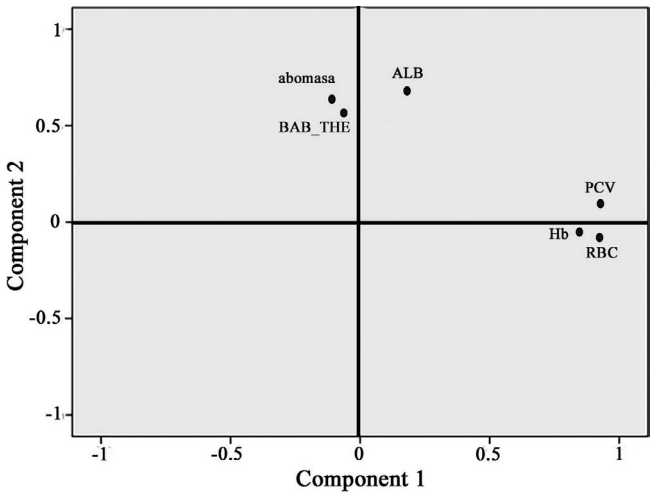

FIGURE 2 Principal component analysis of different risk factors. Component plot: haematological parameters (RBC, Hb and PCV) positively correlated with component 1 and the result of PCR for (BAB_THE) *Babesia*/*Theileria*, (abomasum content) positive abomasum content for nematodes and (ALB) albumin positively correlated with component 2

Out of the second cluster of animals (green circle), we conducted a further analysis, which led to the identification of 5 subgroups corresponding to animals that may have variable levels of phenotypic resistance (Table 7).

Subgroup 1 (*n* = 12): it includes animals with negative *Babesia*/*Theileria* PCR and with presence of nematodes in the abomasum contents. In this subgroup, TWC was > 500 associated with high FEC (FEC > 500). Animals belonging to this subgroup had normal values of RBC and PCV. Ninety-one per cent of the animals had normal values of Hb. Only 66.7% had normal values of albumin and total proteins. In this first subgroup, the percentage of infected females from Béja was significantly higher.

Subgroup 2 (*n* = 9): it includes animals with negative *Babesia*/*Theileria* PCR, presence of nematodes in the abomasum contents, TWC > 500 and low FEC (FEC < 500) with no impact on haematological parameters. All animals had normal values of total proteins and only 5/9 animals had normal values of albumin. In this subgroup, no significant differences were observed according to sex, breed, location or age.

Subgroup 3 (*n* = 81): it includes animals with negative *Babesia*/*Theileria PCR* and presence of nematodes in the abomasum contents. These animals had normal haematological and biochemical parameters. In this third subgroup, percentage of animals belonging to the Cross breed was significantly higher. No significant differences were recorded between age groups.

Subgroup 4 (*n* = 72): it includes animals that are not host susceptible to infection by *Babesia*/*Theileria* and abomasum nematodes. In fact, 71/72 animals had negative *Babesia*/*Theileria* PCR and absence of nematodes in the abomasum contents. All these animals have normal values of RBC and PCV. Only 91.67, 48.61 and 81.94% of animals had normal values of Hb, albumin and total proteins. In this subgroup, the percentage of infected animals aged less than 1 year and belonging to the Noire de Thibar and Queue Fine de l'Ouest was significantly higher.

Subgroup 5 (*n* = 126): Animals in this subgroup are characterized by presence of nematodes in the abomasum contents. Ninety-six per cent of animals had negative *Babesia*/*Theileria* PCR. For haematological parameters, 99.21; 75.40 and 97.62% of animals had normal values of RBC, Hb and PCV respectively. For biochemical parameters, 17.46 and 76.98% of animals had normal values of albumin and total proteins. In this subgroup, the percentage of infected males (51.14%) was significantly higher than females (38.21%) ($p = .04$).

3.3 | Relation between haematological parameters, worm recovery and simple flotation technique results

Multiple correlations showed significant positive correlations between RBC/Hb, RBC/PCV, PCV/Hb and FEC/TWC. Significant

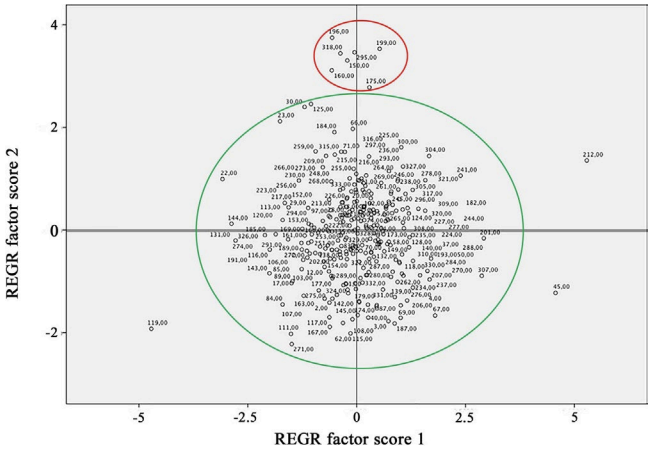

FIGURE 3 Dispersion of observations plots for Principal component analysis. There are 2 clusters of sheep. Cluster one (red circle): sheep with positive *Babesia*/*Theileria* PCR, positive abomasum content for nematodes, with high values of albumin and normal values of haematological parameters (RBC, Hb and PCV). Cluster two (green circle): all other sheep

TABLE 5 Description of sheep clusters obtained by principal component analysis (PCA)

Parameter	Cluster 1	Cluster 2
Babesia:Theileria PCR		
Positive	7/7 (100%)	6/302 (2.01%)
Negative	0/7 (0%)	296/302 (97.99%)
Nematodes in the abomasum contents		
Presence	7/7 (100%)	226/300 (75.76%)
Absence	0/7 (0%)	74/300 (24.24%)
Haematological parameters under threshold values		
Red Blood Cells	0/7 (0%)	2/302 (0.33%)
Haemoglobin	0/7 (0%)	39/299 (12.84%)
Packed cell volume	0/7 (0%)	3/302 (1.00%)
Biochemical parameters under threshold values		
Albumin	0/7 (0%)	149/295 (50.68%)
Total Proteins	0/7 (0%)	46/295 (16.10%)

negative correlations were observed between: TWC/RBC and TWC/Hb (Table 8).

4 | DISCUSSION

In northern Tunisia, climate is favourable for sheep infection by abomasum nematodes, mainly *Haemonchus* spp. In this work, the choice of the geographic area was based on a previous study targeting regions where gastrointestinal parasites, and particularly nematode infection in the abomasum, represents one of the main constraints to small ruminants' production (Akkari et al., 2012). In fact, temperature and rainfall are primordial for the development and survival of infective gastrointestinal worms (Akkari et al., 2012). The parasitic burdens increase with the onset of rains in autumn (Akkari et al., 2012) and our sampling took place during this period (September to December) when prevailing temperatures are still favourable for the development of the studied parasite species. Targeting highly infected areas during optimal periods enhances probabilities to identify traits of resistance amongst sheep.

Infection prevalence of the sheep was estimated to 30.82% by the simple flotation technique. Other authors used the same technique and reported a significantly higher infection prevalence. Indeed, in Spain and Morocco, the infection prevalence was estimated to 87.9% (Martinez-Gonzillez, Diez-Bafios, & Rojo-Vfizquez, 1998) and 68% (Pallargues, Mage, Boukalouch, & Kkalayoune, 2007) respectively. Differences may be caused by variations in the production system and/or differences in the susceptibility of the studied breeds of sheep to infections by GIN.

In comparison to young animals aged less than one year, the infection prevalence by GIN was higher in animals of 12 months and more (p = .001). The sampled sheep were exclusively grazing natural vegetation and as a result, older animals have been more exposed to parasites than younger counterparts. A higher prevalence of GIN infection was found in faeces of Barbarine breed sheep. Such a higher susceptibility of the Barbarine breed could be explained by the fact that the study area is not the homeland of this breed and therefore animals may be experiencing signs of inadaptation to this new epidemiological context. In fact, Barbarine sheep are tradionally found in the meridional central and southern parts of Tunisia where the climate is semi-arid (<350 mm/year) to arid (<150 mm/year) (Ben Salem, Lassoued, & Rekik, 2011).

In concordance with results obtained by simple flotation technique, the highest infection prevalence of the abomasum contents was observed in (i) Barbarine breed, (ii) in Tabarka and Béja regions and (iii) in adult animals aged of 12 months or more. The kappa concordance coefficient between simple flotation technique and worm recovery was 0.21 which is a fair agreement. The fair agreement could be explained by the number and the species of parasites present in the abomasum which are able to influence the result of simple flotation technique (Amarante, 2000). In fact, high values of FEC could be related to several factors including the species of parasites, infection intensity, immunity, sex-ratio of parasites, prepatent period and even the period of the day. For example, in the case of *H. contortus*, one of the main species found in this study, the oviposition rate is very high, ranging between 5,000–15,000 eggs/female/day but only 100 to 200/eggs/female/day are shed for *Trichostrongylus* spp. and *Ostertagia* spp. (Hansen, 1995).

The overall prevalence of *Babesia* and *Theileria* infection was 4.21%. This prevalence was compared to values in the study of Rjeibi, Darghouth, and Gharbi (2016) by PCR which reached 16.3 and 7.8% for *Theileria ovis* and *Babesia ovis* respectively. The higher prevalence obtained in the work of Rjeibi et al. (2016) could be attributed to the sampling location. In fact, authors identified the different piroplasms from three Tunisian bioclimatic zones (north, centre, and south) and they found that *Babesia ovis* was found exclusively in sheep from the centre of Tunisia whereas *Theileria ovis* was found in all regions.

TABLE 6 Characteristics of animals in the two clusters according to principal component analysis results

Parameter	Cluster 1 (*n* = 7)	*p* value	Cluster 2 (*n* = 300)	*p* value
Sex				
Male	1/307 (0.33%)	**.05***	88/307 (28.66%)	**<.001****
Female	6/307 (1.95%)		212/307 (69.06%)	
Breed				
Barbarine	0/307 (0%)	.16	36/307 (11.73%)	**<.001****
Cross breed	4/307 (1.30%)		137/307 (44.63%)	
Noire de Thibar	1/307 (0.33%)		58/307 (18.89%)	
Queue Fine de l'Ouest	2/307 (0.65%)		69/307 (22.48%)	
Location (district)				
Ariana (Ariana)	0/307 (0%)	.58	34/307 (11.07%)	**<.001****
Beja (Beja)	2/307 (0.65%)		51/307 (16.61%)	
Bizerte (Bizerte)	2/307 (0.65%)		26/307 (8.47%)	
Mateur (Bizerte)	0/307 (0%)		50/307 (16.29%)	
Sajnène (Bizerte)	1/307 (0.33%)		38/307 (12.38%)	
Jendouba (Jendouba)	1/307 (0.33%)		48/307 (15.64%)	
Tabarka (Jendouba)	1/307 (0.33%)		24/307 (7.82%)	
Tunis (Tunis)	0/307 (0%)		29/307 (9.45%)	
Age group (years)				
<1	4/307 (1.30%)	.70	236/307 (76.87%)	**<.001****
≥1	3/307 (0.98%)		64/307 (20.85%)	

Note: In bolded characters, statistically significant.

*Significant (.001 ≤ *p* ≤ .05).

**Highly significant (*p* < .001).

In this survey, negative correlations were found between haematological parameters (RBC, Hb and PCV) and TWC. The latter finding is evident since abomasum nematodes such as *H. contortus* are haematophagous and cause significant changes in haematological parameters, mainly anaemia (Al-Quaisy, Al-Zubaidy, Altaif, & Makkawi, 1987). In other studies, the experimental infection of Sahabadi sheep by GIN induced a significant decrease in haemoglobinaemia. In Merino sheep with an infection by 11,000 *H. contortus* L3, the mean values of PCV and haemoglobinaemia decreased progressively. When the infection persisted, PCV and haemoglobin values continued to decline (Allonby & Urquhqrt, 1975).

PCA revealed two animal clusters, the first is composed of a small number of animals (*N* = 7). We consider this small group of animals, with a higher number of females, as possible carriers of some kind of resistance to parasite infection. In this group, 6/7 animals had relatively low TWC (<500). This low burden could be attributed to the resistance of sheep to these parasites, but this hypothesis needs to be confirmed by genetic analyses. There were no Barbarine breed animals in this group. These findings may lend support to our first hypothesis that the sampling location in this study is not the natural agro-ecological niche for animals of this breed, hence showing signs of vulnerability to infection by abomasum nematodes. In this "resistant" group, the number of females was significantly higher (p 0.05) hence suggesting that females may be more resistant than males.

Even if the immunity against *Theileria*/*Babesia* and abomasum parasites is totally different, the cluster *Theileria*/*Babesia*/presence of nematodes in the abomasum contents has been distinguished by the PCA. From this preliminary finding, we could conclude that such resistance has a genetic background. But this conclusion is very preliminary and further studies are needed to confirm this hypothesis and understand its mechanisms.

Overall and based on the various parasitological as well haematological and biochemical parameters of the animals in the clusters and subgroups, our results seem to point out that females of the cross breed may be the most resistant animals to abomasum nematodes. This result is in concordance with other studies since Klein (2000a, 2000b) showed a marked susceptibility of male lambs to GIN's compared to female lambs. This may be attributed to the difference in sex steroid hormone environment (androgens and oestrogens) which is one of the drivers of mammals' immune response (Klein, 2000 a, b). The variation in resistance to abomasum nematodes between breeds has also been largely documented in sheep. For example, In Brazil, Amarante, Bricarello, Rocha, and Gennari, (2004) showed a relative resistance of Santa Ines young male sheep compared to Suffolk and Ile de France sheep. In the highlands of Ethiopia, Getachew et al. (2015) suggested that Menz lambs present a higher level of resistance to *H. contortus*. In the same country, Haile et al. (2002) highlighted that Menz lambs

TABLE 7 Characteristics of animals in the subgroups of cluster 2

Parameter	Subgroup 1 (*n* = 12)	*p* value	Subgroup 2 (*n* = 9)	*p* value	Subgroup 3 (*n* = 81)	*p* value	Subgroup 4 (*n* = 72)	*p* value	Sub-group 5 (*n* = 126)	*p* value
Sex										
Male	0/88 (0%)	**.02***	3/88 (3.41%)	.78	19/88 (21.59%)	.17	21/88 (23.86%)	.97	45/88 (51.14%)	**.04***
Female	12/212 (5.66%)		6/212 (2.83%)		62/212 (29.25%)		51/212 (24.06%)		81/212 (38.21%)	
Breed										
Barbarine	3/36 (8.33%)	.17	2/36 (5.56%)	.25	8/36 (22.22%)	**.006****	5/36 (13.89%)	**.032***	18/36 (50%)	.54
Cross breed	6/137 (4.38%)		2/137 (1.46%)		50/137 (36.50%)		26/137 (18.98%)		53/137 (38.69%)	
Noire de Thibar	3/58 (5.17%)		1/58 (1.72%)		9/58 (15.52%)		18/58 (31.03%)		27/58 (46.55%)	
Queue Fine de l'Ouest	0/69 (0%)		4/69 (5.80%)		14/69 (20.29%)		23/69 (33.33%)		28/69 (40.58%)	
Location (district)										
Ariana (Ariana)	0/34 (0%)	**.005****	1/34 (2.94%)	.98	12/34 (35.29%)	**<.001****	11/34 (32.35%)	**.003****	10/34 (29.41%)	**<.001**
Beja (Beja)	7/51 (13.73%)		2/51 (3.92%)		12/51 (23.53%)		4/51 (7.84%)		26/51 (50.98%)	
Bizerte (Bizerte)	1/26 (3.85%)		1/26 (3.85%)		1/26 (3.85%)		4/26 (15.38%)		19/26 (73.08%)	
Mateur (Bizerte)	0/50 (0%)		1/50 (2%)		15/50 (30%)		15/50 (30%)		9/48 (18.75%)	
Sajnène (Bizerte)	0/38 (0%)		1/38 (2.63%)		2/38 (5.26%)		13/38 (34.21%)			
Jendouba (Jendouba)	2/48 (4.17%)		2/48 (4.17%)		22/48 (45.83%)		13/48 (27.08%)			
Tabarka (Jendouba)	2/24 (8.33%)		0/24 (0%)		15/24 (62.50%)		1/24 (4.17%)		19/50 (38%)	
Tunis (Tunis)	0/29 (0%)		1/29 (3.45%)		2/29 (6.90%)		11/29 (37.93%)			
Age group (years)										
<1	8/236 (3.39%)	.3	6/236 (2.54%)	.37	63/236 (26.69%)	.81	63/236 (26.69%)	**.036***	22/38 (57.89%)	
≥1	4/64 (6.25%)		3/64 (4.69%)		18/64 (28.13%)		9/64 (14.06%)		6/24 (25%)	

Note: In bolded characters, statistically significant at 5% threshold.

*Significant (.001 ≤ *p*≤.05).

**Highly significant (*p* < .001).

TABLE 8 Matrix correlation between haematological and quantitative coproscopic parameters

		FEC	RBC	Hb	PCV	TWC
FEC	Pearson correlation	1				
	p (bilateral)					
RBC	Pearson correlation	−.015	1			
	p (bilateral)	.795				
Hb	Pearson correlation	−.011	**.664****	1		
	p (bilateral)	.857	**.000**			
PCV	Pearson correlation	−.014	**.852****	**.672****	1	
	p (bilateral)	.814	**.000**	**.000**		
TWC	Pearson correlation	**.148***	**−.174****	**−.116***	**−.100**^{t}	1
	p (bilateral)	**.012**	**.002**	**.043**	**.080**	

Note: In bolded characters, statistically significant at 5% threshold.

Abbreviations: FEC, faecal egg count; RBC, red blood cells; Hb, haemoglobinaemia; PCV, packed cell volume; TWC, total worm count; *p*, probability; ^{t}: tendency.

*Significant ($.001 \leq p \leq .05$).

**Highly significant ($p < .001$).

acquire the ability to resist and tolerate endoparasite infections better than Horro lambs.

A surprising finding revealed by this study is that animals of the Barbarine breed seem to show low resistance to abomasum nematodes. This is not consistent with what has been reported by Ben Salem et al. (2011) in their review depicting this breed as being very resistant to environmental stressors and characterized by a strong metabolic and digestive adaptation to harsh environmental conditions. The presence of higher parasitological indicators and impact on haematological parameters should not allow us to conclude that this breed is not adapted to these parasites. Indeed, this breed could also be resilient to these pathogens. It means that they are infected (sometimes highly infected) but, thanks to a parsimony strategy, the immune system of infected animals develops only a mild immune reaction (Sigal, 2003). Despite harbouring a greater nematode burden and greater FEC, resilient animals perform well in the presence of the parasite (Morris et al., 1997). Differences between resilient and resistant animals appear in the time of occurrence of immune response. Differences between resilient and resistant animals are expressed in the immune response installation delay. In a study that compared the delay of immunity installation against gastro-intestinal nematodes parasites in resistant and resilient selection lines of Romney lambs, authors found that resistant animals developed immunity earlier and these animals were more physiologically mature than resilient animals (Hamie, McAnulty, Logan, Lundberg, & Greer, 2019).

5 | CONCLUSIONS

Our results to screen for possible indicators of resistance to abomasum nematodes in autochthonous Tunisian sheep breeds are preliminary and we are not aware of such a similar work in the South Mediterranean. To summarize and when it comes to possible resistance to abomasum nematodes, females from the cross breed can be spotted as being possible carriers of some kind of resistance. This may reflect a genetic basis for resistance, but further studies are needed to confirm this hypothesis. Phenotypic measurements are an important prerequisite to help understand forms of the resistance expressed by animals and identifying genomic regions and pathways associated with resistance to gastrointestinal parasites. When candidate genes related to resistance are found, they may help in the selection of animals with higher resistance to parasites.

ACKNOWLEDGEMENTS

This work was supported by the Laboratoire d'Épidémiologie des Infections Enzootiques des Herbivores en Tunisie: Application à la Lutte (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique, Tunisia) (LR16AGR01). This study was also partly supported by the CGIAR Research Program on Livestock (CRP Livestock).

CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTION

Mariem Rouatbi: Data curation; Investigation; Writing-original draft. Rihab Romdhane: Investigation. Faten Bouaicha: Investigation. Rahma Saddem: Investigation. Limam Sassi: Investigation. Mokhtar Dhibi: Formal analysis. Mourad Rekik: Conceptualization; Funding acquisition; Writing-review & editing. Aynalem Haile: Conceptualization; Resources; Writing-review & editing. Joram Mwacharo: Methodology; Writing-original draft. Barbara Rischkowsky: Project administration; Writing-review & editing. Mohamed Darghouth: Conceptualization; Writing-review & editing. Mohamed Gharbi: Conceptualization; Supervision; Writing-review & editing.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required for this study.

ORCID

Mariem Rouatbi https://orcid.org/0000-0003-0117-0266
Mohamed Gharbi https://orcid.org/0000-0002-7074-2021

REFERENCES

Akkari, H., Gharbi, M., & Darghouth, M. A. (2011). Infestation of tracer lambs by *Fasciola hepatica* in Tunisia: Determining periods for strategic anthelmintic treatments. *Scientific and Technical Review - OIE*, *30*, 917–929. https://doi.org/10.20506/rst.30.3.2084

Akkari, H., Gharbi, M., & Darghouth, M. A. (2012). Dynamics of infestation of tracers lambs by gastrointestinal helminths under a traditional management system in the North of Tunisia. *Parasite*, *19*, 407–415. https://doi.org/10.1051/parasite/2012194407

Akkari, H., Rtibi, K., B'chir, F., Rekik, M., Darghouth, M. A., & Gharbi, M. (2014). In vitro evidence that the pastoral *Artemisia campestris* species exerts an anthelmintic effect on *Haemonchus contortus* from sheep. *Veterinary Research Communications*, *38*, 249–255. https://doi.org/10.1007/s11259-014-9609-y

Allonby, E. W., & Urquhqrt, G. M. (1975). The epidemiology and pathogenic significance of haemonchosis in a Merino flock in East Africa. *Veterinary Parasitology*, *1*, 129–143. https://doi.org/10.1016/0304-4017(75)90015-1

Al-Quaisy, H. H., Al-Zubaidy, A. J., Altaif, K. I., & Makkawi, T. A. (1987). The pathogenicity of haemonchosis in sheep and goats in Iraq. Clinical, parasitological and haematological findings. *Veterinary Parasitology*, *24*, 221–228. https://doi.org/10.1016/0304-4017(87)90043-4

Al-Shaibani, I. R. M., Phulan, M. S., & Shiekh, M. (2009). Anthelmintic activity of *Fumaria parviflora* (*Fumariaceae*) against gastrointestinal nematodes of sheep. *International Journal of Agriculture and Biology*, *11*, 431–436.

Amarante, A. F. T. (2000). Relationship between faecal egg count and total worm counts in sheep infected with gastrointestinal nematodes. *The Revista Brasileira De Parasitologia Veterinária*, *9*, 45–50.

Amarante, A. F. T., Bricarello, P. A., Rocha, R. A., & Gennari, S. M. (2004). Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. *Veterinary Parasitology*, *120*, 91–106. https://doi.org/10.1016/j.vetpar.2003.12.004

Ben Salem, H., Lassoued, N., & Rekik, M. (2011). Merits of the fat-tailed Barbarine sheep raised in different production systems in Tunisia: Digestive, productive and reproductive characteristics. *Tropical Animal Health and Production*, *43*, 1357–1370. https://doi.org/10.1007/s11250-011-9863-8

Bishop, S. C. (2012). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal*, *6*, 741–747. https://doi.org/10.1017/S1751731111000681

Bishop, S. C., & Stear, M. J. (2003). Modeling of host genetics and resistance to infectious diseases: Understanding and controlling nematode infections. *Veterinary Parasitology*, *115*, 147–166. https://doi.org/10.1016/s0304-4017(03)00204-8

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al Revisited. *Journal of Parasitology*, *83*, 575–583. https://doi.org/10.2307/3284227

Chebbi, H. E. (2019). Rapport de synthèse sur l'agriculture en Tunisie, Projet d'appui à l'initiative ENPARDMEDITERRANEE, 98 pp.

Dean, A. G., Arner, T. G., Sunki, G. G., Friedman, R., Lantinga, M., Sangam, S., … Fagan, R. F. (2011). *Epi Info™ A database and statistics program for public health professionals*. Atlanta, GA, USA: CDC.

Deeba, F., Muhammad, G., Iqbal, Z., & Hussain, I. (2009). Survey of ethnoveterinary practices used for different ailments in dairy animals in peri-urban areas of Faisalabad (Pakistan). *International Journal of Agriculture and Biology*, *11*, 535–541.

Euzeby, J. (1982). *Diagnostic expérimental des helminthoses animales. Tome 2*. Paris: Information Technique des Services Vétérinaires Minagri, 364 pp.

Getachew, T., Alemu, B., Sölkner, J., Gizaw, S., Haile, A., Gosheme, S., & Notter, D. R. (2015). Relative resistance of Menz and Washera sheep breeds to artificial infection with *Haemonchus contortus* in the highlands of Ethiopia. *Tropical Animal Health and Production*, *47*, 961–968. https://doi.org/10.1007/s11250-015-0815-6

Gharbi, M., Zribi, L., Jedidi, M., Chakkhari, H., Hamdi, S., R'hayem, S. S., … Darghouth, M. A. (2013). Prevalence of *Toxoplasma gondii* infection in Tunisian sheep. *Bulletin de la Société de Pathologie Exotique*, *106*, 184–187. https://doi.org/10.1007/s13149-013-0290-4

Gubbels, J. M., de Vos, A. P., Van der Weide, M., Viseras, J., Schouls, L. M., de Vries, E., & Jongejan, F. (1999). Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridisation. *Journal of Clinical Microbiology*, *37*, 1782–1789.

Haile, A., Tembely, S., Anindo, D. O., Mukasa-Mugerwa, E., Rege, J. E. O., Yami, A., & Baker, R. L. (2002). Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep. *Small Ruminant Research*, *44*, 247–261. https://doi.org/10.1016/S0921-4488(02)00080-9

Hamie, J. C., McAnulty, R. W., Logan, C. M., Lundberg, S., & Greer, A. W. (2019). Comparison of the timing of development of immunity in Romney lambs from resistant and resilient selection lines. *Veterinary Parasitology*, *270*, 49–55. https://doi.org/10.1016/j.vetpar.2019.04.014

Hansen, J. (1995). *Épidémiologie, diagnostic et prophylaxie des helminthiases des ruminants domestiques*. Rome: Organisation des Nations Unies pour l'alimentation et l'agriculture, 176 pp.

Hussain, A., Khan, M. N., Iqbal, Z., & Sajid, M. S. (2008). An account of the botanical anthelmintics used in traditional veterinary practices in Sahiwal district of Punjab. *Pakistan. Journal of Ethnopharmacology*, *119*, 185–190. https://doi.org/10.1016/j.jep.2008.06.034

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, F. M. N., & Afaq, M. (2006). Anthelmintic resistance: The state of play revisited. *Life Sciences*, *79*, 2413–2431. https://doi.org/10.1016/j.lfs.2006.08.010

Kaplan, R. M. (2009). Anthelmintic treatment in the era of resistance. In D. Anderson, & M. Rings (Eds.), *Food animal practice* (5th ed., pp. 470–478). St. Louis, MO: Saunders.

Klein, S. L. (2000a). Hormones and mating system affect sex and species differences in immune function among vertebrates. *Behavioural Processes*, *51*, 149–166. https://doi.org/10.1016/s0376-6357(00)00125-x

Klein, S. L. (2000b). The effects of hormones on sex differences in infection: From genes to behavior. *Neuroscience and Biobehavioral Reviews*, *24*, 627–638. https://doi.org/10.1016/s0149-7634(00)00027-0

Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics*, *33*, 159–174. https://doi.org/10.2307/2529310

Martinez-Gonzillez, B., Diez-Bafios, N., & Rojo-Vfizquez, F. A. (1998). An epidemiological study of gastrointestinal parasitism in dairy sheep flocks in Leon (NW Spain). *Small Ruminant Research*, *27*, 25–30. https://doi.org/10.1016/S0921-4488(97)00008-4

Mohamed-Brahmi, A., Khaldi, R., & Khaldi, G. (2010). *L'élevage ovin extensif en Tunisie : disponibilités alimentaires et innovations pour la valorisation des ressources fourragères locales*. Montpellier, France: ISDA, 12 pp.

Morris, C. A., Vlassoff, A., Bisset, S. A., Baker, R. L., West, C. J., & Hurford, A. P. (1997). Responses of Romney sheep to selection for resistance or susceptibility to nematode infection. *Animal Science*, *64*, 319–329. https://doi.org/10.1017/S1357729800015897

Pallargues, T., Mage, C., Boukalouch, A., & Kkalayoune, K. (2007). Étude épidémiologique du parasitisme digestif et pulmonaire des ovins au Maroc. *Annales de Médecine Vétérinaire*, *151*, 1–5.

Raynaud, J. P. (1970). Étude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie Humaine et Comparée*, *45*, 321–342. https://doi.org/10.1051/parasite/1970453321

Rekik, M., Aloulou, R., & Ben Hamouda, M. (2005). Small ruminant breeds of Tunisia. In L. Iñíguez (Ed.), *Characterisation of small ruminant breeds in West Asia and North Africa, Vol. 2. North Africa* (pp. 91–140). Aleppo, Syria: International Center for Agricultural Research in the Dry Areas (ICARDA).

Rjeibi, M. R., Darghouth, M. A., & Gharbi, M. (2016). Prevalence of *Theileria* and *Babesia* species in Tunisian sheep. *Onderstepoort Journal of Veterinary Research*, *83*, e1–e6. https://doi.org/10.4102/ojvr.v83i1.1040

Schwartz, D. (1993). *Méthodes statistiques à l'usage des médecins et des biologistes* (3ème éd.). Paris, France: Flammarion.

Sigal, L. H. (2003). Molecular biology and immunology for clinicians 24: Phylogeny of the immune system. *Journal of Clinical Rheumatology*, *9*, 200–206. https://doi.org/10.1097/01.RHU.0000073611.96104.8e

Sindhu, Z. U. D., Iqbal, Z., Khan, M. N., Jonsson, N. N., & Siddique, M. (2010). Documentation of ethno-veterinary practices used for treatment of different ailments in selected a hilly area of Pakistan. *International Journal of Agriculture and Biology*, *12*, 353–358.

Soulsby, E. J. L. (1983). *Helminths, Arthropods, and Protozoa of Domesticated Animals* (7th ed.). London: The English Language Book Society and Bailliere Tindall, 809 pp. bailliere sans accent, rien entre Baillire et Tindall sauf l'espace.

Taylor, M., Coop, R., & Wall, R. (2007). *Veterinary parasitology* (3rd ed.). Oxford: Blackwell publishing.

Toma, B., Dufour, B., Sanaa, M., & Bénet, J. J. (2007). *Epidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures*. Paris: Association pour l'Etude de l'Epidémiologie des Maladies Animales, 696 pp.

Waller, P. J. (1997a). Sustainable helminth control of ruminants in developing countries. *Veterinary Parasitology*, *71*, 195–207. https://doi.org/10.1016/s0304-4017(97)00032-0

Waller, P. J. (1997b). Nematode parasite control of livestock in the tropics/subtropics: The need for novel approaches. *International Journal for Parasitology*, *27*, 1193–1201. https://doi.org/10.1016/s0020-7519(97)00117-3

How to cite this article: Rouatbi M, Romdhane R, Bouaicha F, et al. Individual variability among autochthonous sheep in Northern Tunisia to infection by abomasum nematodes and *Babesia*/*Theileria* parasites. *Vet Med Sci*. 2020;00:1–12. https://doi.org/10.1002/vms3.310