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Changes in hematological status depending on reproductive stage of ewes naturally infected with internal parasites in North-Eastern Algeria

Cambios en el estado hematológico en función de la fase reproductiva de ovejas infectadas naturalmente por parásitos internos en el noreste de Argelia

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ABSTRACT

In Algeria, sheep farming is still largely traditional, and ewes are subject to various pathologies, particularly parasitic diseases leading to reproductive failure and production losses. To assess the influence of internal parasites (helminths and protozoa) and their interaction with the different phases of reproduction and season on hematological parameters, fecal and blood samples (n = 89) were taken from ewes during the period from the end of gestation to 3 months after parturition, two weeks apart. Animals were reared under a traditional pasture-based farming system in a Region of Northeastern Algeria characterized by a Mediterranean climate. Parasitic coproscopy results showed the consistent presence of digestive strongyles, Strongyloides, Trichuris ovis, Moniezia spp., Fasciola hepatica, and Coccidia. Hematological parameters, mainly hematocrit (HCT), hemoglobin (HGB), and erythrocytes (RBC), showed lower levels in anemic ewes throughout the study period. These hematological effects became significantly prevalent in 91% of the animals (P<0.05) during the third month postpartum (p.p.). The use of a mixed-effects model confirmed the significant hematological effect (P<0.05) of internal parasitism, which was amplified significantly by its interaction with the reproduction stage for HCT and RBC (P<0.01). Monitoring of blood biological constants in ewes during the critical reproductive stages seems to provide valuable data to improve their welfare and resistance to potential internal parasites.

Key words: Ewes; hematology; internal parasites; peripartum

RESUMEN

En Argelia, la ganadería ovina sigue siendo en gran medida tradicional, y las ovejas están sujetas a diversas patologías, en particular enfermedades parasitarias que provocan fallos reproductivos y pérdidas de producción. Para evaluar la influencia de los parásitos internos (helmintos y protozoos) y su interacción con las diferentes fases de la reproducción y la estación del año sobre los parámetros hematológicos, se tomaron muestras fecales y sanguíneas (n = 89) de ovejas durante el periodo comprendido entre el final de la gestación y 3 meses después del parto, con un intervalo de dos semanas. Los animales se criaban en un sistema tradicional de pastoreo en una región del noreste de Argelia caracterizada por un clima mediterráneo. Los resultados de la coproscopía parasitaria mostraron la presencia constante de estróngilos digestivos, Strongyloides, Trichuris ovis, Moniezia spp., Fasciola hepatica y Coccidia. Los parámetros hematológicos, principalmente hematocrito (HCT), hemoglobina (HGB) y eritrocitos (RBC), mostraron niveles más bajos en las ovejas anémicas durante todo el periodo de estudio. Estos efectos hematológicos se hicieron significativamente incidentes en el 91 % de los animales (P<0,05) durante el tercer mes posparto (p.p.). El uso de un modelo de efectos mixtos confirmó el efecto significativo (P<0,05) del parasitismo interno en el estado hematológico, que se amplificó significativamente por su interacción con la fase de reproducción para el HCT y el RBC (P<0,01). La monitorización de las constantes biológicas sanguíneas de las ovejas durante las fases reproductivas críticas parece aportar datos valiosos para mejorar su bienestar y su resistencia a posibles parásitos internos.

Palabras clave: Ovejas; hematología; parásitos internos; periparto



INTRODUCTION

Sheep (Ovis aries) play a key role in the Algerian agricultural economy due to their dominant proportion in the National herd (78%) with approximately 29.4 million head, including 18.4 million ewes in 2019 as reported by the Algerian Ministry of Agriculture and Rural Development [1]. Sheep farming is subject to considerable pathology, including those related to the presence of internal parasites that cause high mortality, decreased production, and significant overall economic losses [2, 3, 4].

In the Algerian Coastal Region characterized by a Mediterranean climate, contrary to the steppe area with a semiarid climate $[\underline{5},\underline{6},\underline{7}]$, the internal parasitism of sheep is not well documented. Its study mainly involves surveys in slaughterhouses $[\underline{8}]$. In North Africa, internal parasites develop under common climatic conditions and it has been suggested that some of their variants, such as the trematode Fasciola hepatica, have a common origin and spread in three Mediterranean Countries (Algeria, Tunisia, and Spain) due to movements of infected animals $[\underline{9}]$.

Hematology of infected animals is a very sensitive indicator of the degree of parasite–induced lesions, particularly liver lesions, which disrupt metabolic processes vital to the normal health and optimal productivity of the animals [10, 11]. The assessment of hematological data in sheep is an essential complement to the detection of the presence of parasites [12, 13]. Furthermore, blood loss generated by parasites can lead to anemia and promote hematological and biochemical alterations if acute [14].

The present study aims to investigate, in a traditional Algerian breeding system, the hematological effects of some potential internal parasites that develop in the Mediterranean climate in ewes at critical stages of reproduction.

MATERIALS AND METHODS

Study area

The present study was carried out in El-Tarf Province, a littoral Region located in North-Eastern Algeria with a Mediterranean climate (36°75′N; 7°93′E) during the rainy months of the year (from October to April). The temperature varies throughout the year between 10° and 36°C, with average annual precipitation of about 600 mm. According to the Köppen-Geiger classification, the climate is Csa [15].

Animals

Fourteen ewes of autochthon breeds (Ouled-Djellal and Berber breeds), aged between 2 and 5 years from a private farm maintained under traditional and semi-intensive management systems were eartagged. The ewes were weekly monitored at different reproductive stages (late pregnancy, early, and mid-lactation). The ewes pasture daily but on rainy days, they were fed with hay and straw and supplemented intermittently with concentrate when available. The deworming was indiscriminately performed by a Veterinary practitioner in response to the owner's request. From the second month postpartum and following the death of two ewes, the number of subjects followed was reduced to twelve. This resulted in the missing values indicated below in the statistical description.

Blood samples and hematological parameters measurement

For each ewe, the hematological status was established from Ethylenediaminetetraacetic acid (EDTA) –blood samples (10 mL) collected at 8:00 am once during the antepartum (AP), one to three weeks before lambing, and twice a month from the parturition to the 3rd month postpartum (FIG. 1).

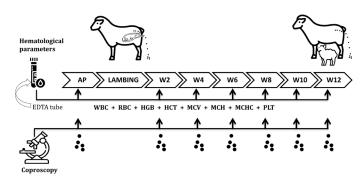


FIGURE 1. Study sampling design for coproscopic examination and blood parameters assessment

The hematological indicators monitored were White Blood Cell count (WBC), erythrocytes parameters mainly Red Blood Cell count (RBC), Hemoglobin concentration (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and platelet parameters (PLT) using an Auto Hematology Analyzer (BC–5380, Shenzhen Mindray Bio-medical Electronics Co, Ltd, China). Females were categorized into anemic and non-anemic groups according to their hematocrit values lower than the threshold value (27%) reported by Byers–Stacey and Kramer [16] (TABLE I).

TABLE I
Reference values of assessed hematological parameters in ewes

Hematological parameters (unit)	Range*	Mean*
WBC (×10³·µL⁻¹)	4 - 8	12.0
RBC (×106·µL⁻¹)	9 – 15	12.0
HGB (g·dL⁻¹)	9 - 15	11.5
HCT (%)	27 - 45	35.0
MCV (flv)	28 - 40	34.0
MCH (pg)	8 – 12	10.0
MCHC (%)	31 - 34	32.5
PLT (×10 ³)	800 - 1,100	500.0

^{*:} Reference values reported by Byers-Stacey and Kramer [16]

Fecal samples and coproscopic examination

Once during antepartum and fortnightly from lambing to weaning, fecal samples were collected directly from the rectum of each ewe in labeled plastic bags (FIG. 1).

Fecal egg counts (FECs) of internal parasites (gastrointestinal nematodes, *Coccidia*, *Moniezia*, and *Fasciola hepatica* eggs) were estimated using the Modified Wisconsin Sugar Flotation (Sheather's solution, specific gravity 1.27) [17]. The identification of internal parasites' eggs was based on the morphological criteria reported by Bussiéras and Chermette [18]. For worm burdens and coccidiosis, the FEC was considered low for less than 10 eggs/oocysts per gram (EPG/OPG) and moderate for 11 to 50 eggs/oocysts. A high level of worm burden requiring anthelmintic administration was defined at a threshold of 50 EPG. According to these FEC levels, females were assigned to three groups, Low, Moderate, and High levels both for worm burdens and *Eimeria* spp. The presence of liver fluke eggs in the fecal sample led us to define two other ewes' groups, fasciolosis "Absence "and "Presence" [17, 19].

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics 26 software. The mixed-effects model for repeated measurements was used to measure the model for the hematological variation according to the anemia status, season (months), and internal parasites eggs shedding level (FEC level). These factors and their interaction with the reproductive stage (Time) were considered as fixed effects (missing values were not imputed).

Independent samples t-tests were performed to compare measures between the two groups at the limit reference values, antepartum, weeks 2, 4, 6, 8, 10, and 12. Overall within-group variations in measures across reproductive stages (weeks) were tested using repeated-measures ANOVA. Furthermore, a chi-squared (χ^2) test was performed for comparison between the proportions of the group's categories.

To compare the distribution of internal parasite parameters (FECs) across categories of groups, non-parametric tests for independent samples were used. Anemia and fasciolosis categories via Mann-Whitney U Test; reproductive stage, season, Worm and Coccidian FEC levels through Kruskal-Wallis Test for one way ANOVA for k sample. In addition, Spearman's rho correlation was used to establish the relationship between these parameters and studied factors. The threshold value for all these statistical tests was 5%.

RESULTS AND DISCUSSIONS

The micro-coproscopic study made it possible to follow the evolution of female infection by internal parasites according to the stage of reproduction and season.

As shown in TABLE II, gastrointestinal nematodes, mainly strongyles, shed a low number of eggs (< 10 EPG) during most of the reproductive stages; the only significant increase in egg shedding but not exceeding the moderate level of 50 EPG has occurred at the second week postpartum and no ewes had a high-level FEC.

These changes in the level of nematode egg shedding corresponding to a periparturient rise were revealed by the second peak observed during late Autumn-early Winter (November-December) and which were also reported in North Tunisia [20]. This phenomenon which began about two weeks before lambing, and continued up to eight weeks after lambing [21], was assigned to the physiological changes characterizing the peripartum period. The endocrine and metabolic effects of this transition period are closely associated with leptin and cortisol profiles in response to the mobilization of fat and protein

reserves on the anti-parasite immune system relaxation of the host and on worm egg count had been blamed [22].

The level of excretion of *Eimeria* oocysts was often low, it increased from the second–week p.p.; its incidence for a moderate level increased insignificantly in the third month postpartum in 40% of the animals and its highest level was never reached during the study. The presence of *Fasciola* egg in fecal material was observed from 15 days after parturition at the highest prevalence value (TABLE II). According to the chi–square test, the distribution of groups did not seem to be wholly affected by the reproductive stage.

Worm eggs have been consistently disposed of at a low level without ever reaching the high level (TABLE II) except for a significant prevalence rise of ewes with moderate EPG in January (66.7%). As well, an increase in ewes with moderate *Eimeria* oocysts count and a significant presence of Fasciolosis in 61.9% of fecal samples were observed in January (TABLE II). In the subsequent month (February), a marked disappearance of ewes shed a moderate level of coccidian FEC and those excreting *Fasciola* egg was noted. With the arrival of Spring, these categories reappeared over time.

Through the overall comparison of the categories, a significant seasonal variation in the proportions of the groups was recorded for the worm's FEC level (P<0.05) and Fasciolosis (P<0.001).

In the study area, a temperature ranging from 10° to 36° C during the whole year is considered generally favorable for the development of trichostrongylid larvae from egg to L3 infective larva [23]. This climatic–seasonal effect was also verified by the Spring rise in ewes that shed worm eggs and the Spring reappearance of ewes with moderate coccidian FEC levels and Fasciola eggs.

The permanent presence of internal helminths during the rainy and cold seasons (late Autumn and Winter) and the Spring seems related to the climatic condition of this Mediterranean area, in agreement with that reported in North Tunisia by Akkari et al. [20]. Under this temperate climate, the egg count of gastrointestinal helminths showed a gradual increase from January with a peak in May and June due to the ingestion of infective larvae during the rainy and cold seasons. Strongylid eggs were considered cold tolerant, and the succession between low and higher temperatures could stimulate their hatching [23].

For the Fasciola genus, its presence seems surprising given the gravity of Sheather's solution (1.27), which is not the best choice for demonstrating Fasciola hepatica eggs using the floatation method [24]. This suggests an incidence of infection by Fasciola which could have been estimated at higher rates if a solution allowing the flotation of heavy eggs of this trematode had been used. The seasonal occurrence of this trematode could be related to the life-cycle of its intermediate host, the freshwater snail Galba truncatula. The natural infection of this snail was recorded during Winter and Spring from December (2.3%) to May (4.8–9.3%) in the Algerian Coastal Region by Mekroud et al. [25].

The results obtained from hematology tests showed a significant prevalence of anemia in the studied flock, where 50% of the animals were anemic throughout the study and reaching 91% (P<0.05) in the third month postpartum (TABLE II). These results are consistent with those observed by David et al. [26] where they reported significant variations across the gestational period with a decrease in mean corpuscular volume (MVC) and lowest values of hemoglobin concentration (HGB), and corpuscular hemoglobin concentration (MCHC) near the birth date.

TABLE II

Proportions of internal parasites and anemia categories (frequencies) according to the reproductive stage and season

		W	Worm FEC level (%)			Coccidia FEC level (%)			Fasciolosis (%)		Anemia Group (%)	
Factor		Low	Moderate	High	Low	Moderate	High	Absence	Presence	Anemic	Non-anemic	
	AP	100	0	0	100	0	0	100	0	64.3	35.7	
	W2	33.3	44.4	22.2	55.6	44.4	0	44.4	55.6	50	50	
	W4	83.3	8.3	8.3	50	41.7	8.3	83.3	16.7	57.1	42.9	
	W6	66.7	33.3	0	77.8	11.1	11.1	55.6	44.4	66.7	33.3	
Reproductive stage (Time)	W8	75	25	0	25	58.3	16.7	91.7	8.3	58.3	41.7	
stage (Time)	W10	60	40	0	40	50	10	50	50	90.9	9.1	
	W12	66.7	25	8.3	25	50	25	66.7	33.3	50	50	
	χ^2	13.09			14.41			10.33		5.83		
	Р		0.36			0.28			0.11		0.44	
Season (Months)	ОСТ									42.9	57.1	
	NOV	100	0	0	75	25	0	100	0	50	50	
	DEC	91.7	0	8.3	58.3	16.7	25	83.3	16.7	36.8	63.2	
	JAN	19	66.7	14.3	33.3	42.9	23.8	38.1	61.9	70.8	29.2	
	FEB	81.8	18.2	0	36.4	63.6	0	100	0	63.6	36.4	
	MAR	93.3	6.7	0	53.3	46.7	0	73.3	26.7	100	0	
	APR	75	25	0	50	50	0	50	50	50	50	
	χ^2	34.72			14.00			17.96		16.4		
	Р		0.000			0.17			0.003		0.012	

Across months of study, anemia that presents the six months of study (TABLE II) had significantly decreased to affect 36.8% of the flock in Winter (December). Subsequently, it continued to increase until affecting all studied animals as shown by a significant peak in March (100%), thus a significant seasonal variation in the proportions of anemic and non-anemic ewes was noted (P<0.05).

The results of non–parametric tests used to compare the distribution for FEC count of internal parasite species across categories of groups are presented in TABLE III. Anemia status seems to be related to changes in the FEC count of Moniezia and Eimeria (P<0.05) but not to that of another genus of internal parasites (P>0.05). It appears that egg shedding of the Nematode species, except Trichuris ovis, as well as that of Moniezia and Fasciola, were significantly different between months of study (P<0.01). Among all studies genus of internal parasites, only Strongyloides FEC was distributed differently across the reproductive stage (Time), but this did not indicate by significant correlation (rs = 0.14, P>0.05).

The presence of Strongyloides and Trichuris eggs in fecal samples appears generally incidental because of their susceptibility to the anthelmintics used to treat the more important Nematode species such as Haemonchus [23]. These could explain the insignificant seasonal changes in Trichuris egg excretion and the low significance of Strongyloides FEC distribution across reproductive stages.

FIG. 2 demonstrates that leucocyte count (WBC) had exceeded the threshold value (12×10^3 cells· μ L-1) both in anemic and non-anemic ewes from week 2 to week 10 p.p. A single significantly highest value of WBC in non-anemic ewes was recorded during the weaning period (week 8) in comparison with a normal value measured at the same

TABLE III
Distribution tests for internal parasites FEC following to the studied factors

Factor of grouping	Anen	nia	Reprodu stage (T		Season (Month)	
	Test ^a Sig.	rs	Test ^b Sig.	rs	Test ^b Sig.	rs
Nematode worms	0.59	0.07	0.15	-0.01	0.00*	-0.08
Strongyloides	0.46	0.09	0.04*	0.14	0.00*	0.14
Nematodirus	0.35	0.12	0.08	-0.16	0.02*	-0.21
Trichuris	0.99	0.00	0.64	-0.10	0.55	-0.13
Moniezia	0.011*	0.32**	0.19	-0.22	0.00*	-0.45**
Eimeria	0.026*	0.28*	0.34	0.26*	0.54	-0.01
Fasciola	0.492	-0.09	0.15	0.06	0.00*	0.03

^a: comparison of variables distribution across groups using Mann–Whitney U Test.

period in anemic ewes (FIG. 2A). This could be attributed, in the early postpartum period, to the effect of cortisol which triggers delivery $[\underline{27}]$ and afterward to the impact of the parasite infections.

The change in leucocytes number was only influenced by the interaction of the reproductive stage with *Eimeria* FEC level (TABLE IV). This could be related to the release pattern of coccidia which impairs the immune system and reduces the count of WBC following

b: comparison of variables distribution across groups using Kruskal Wallis Test. rs: Spearman's rho correlation with factor of grouping. **: significant at the 0.01 level.

^{*:} significant at the 0.05 level

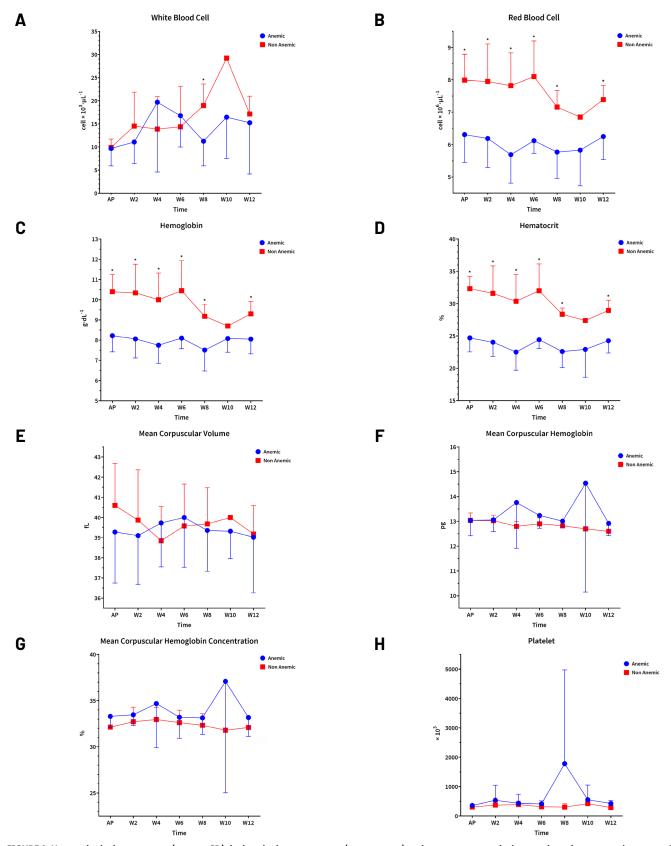


FIGURE 2. Hematological parameters (mean ± SD) during the late pregnancy (antepartum) and postpartum weeks in anemic and non-anemic groups. *: significant difference at P<0.05 between non-anemic ewes and those with anemia using "t" test

TABLE IV

F-tests of fixed effects of studied factors and interactions of internal parasites FEC levels with the reproductive stages (time) in the mixed model for hematological parameters

Factors		Time	Season	GIN	Cocc.	Fasc.	Time × GIN	Time × Cocc.	Time × Fasc.
WBC	F	2.17	2.57	2.51	0.29	0.12	1.35	2.53	0.36
	Sig.	0.15	0.06	0.12	0.59	0.73	0.25	0.045	0.70
RBC	F	16.45	11.78	22.56	0.02	0.11	72.70	9.39	15.17
	Sig.	0.00	0.00	0.00	0.90	0.74	0.00	0.00	0.00
	F	19.09	6.03	1.74	0.16	8.03	4.43	3.90	2.29
HGB	Sig.	0.00	0.00	0.20	0.69	0.01	0.04	0.01	0.11
НСТ	F	21.39	11.15	19.67	0.02	0.02	91.01	9.43	14.05
	Sig.	0.00	0.00	0.00	0.90	0.89	0.00	0.00	0.00
	F	0.45	8.36	15.25	1.06	11.10	6.71	2.51	9.20
MCV	Sig.	0.51	0.00	0.00	0.31	0.00	0.01	0.048	0.00
МСН	F	8.10	12.22	155.48	0.39	8.07	50.41	1.08	31.20
	Sig.	0.01	0.00	0.00	0.54	0.01	0.00	0.39	0.00
МСНС	F	1.16	30.44	175.77	0.37	16.18	56.54	0.61	28.49
	Sig.	0.29	0.00	0.00	0.55	0.00	0.00	0.69	0.00
PLT	F	9.09	0.65	20.09	0.48	0.77	14.11	4.00	0.10
	Sig.	0.01	0.63	0.00	0.50	0.40	0.00	0.01	0.90

Sig.: P-value of F-test for fixed effects which are significant when P<0.05. Anemia: anemia status; Time: reproductive stages; GIN: gastrointestinal nematodes FEC levels; Cocc.: Coccidia FEC levels; Fasc.: Fasciola FEC levels

the infection by digestive strongyles [28] as observed during the antepartum. During this period, prenatal stress mediated by Cortisol [29] and hormonal changes such as Progesterone [30] levels may affect the immune system of pregnant ewes. In addition, it has reported that relative concentrations of eosinophil were highest in the blood of pregnant ewes and reduced dramatically upon parturition, as is characteristic of a stress response [31]. On the contrary, according to Panousis et al. [32], the reproductive stage of the animals does not seem to affect WBC.

At each period of sampling, both during late pregnancy, early postpartum, or weaning except week 10 when most ewes were anemic (91%), a significant difference (*P*<0.05) in red blood cell count (FIG. 2B), hemoglobin concentration as well as hematocrit percentage (FIG. 2C and FIG. 2D) was noted.

These parameter values were highest in non-anemic ewes in comparison with those of anemic ewes. RBC counts were lower than the minimum limit value (9 × 10 cells· μ L-1) regardless of the anemic status of the ewes during pregnancy, before or after parturition and which may be largely due during the gestation period to the physiological anemia of gestation and combined with other factors including internal parasitism such as *Haemonchus contortus* (hematophagy and leakage of blood from the site of attachment of parasites) [11, 13, 33]. In contrast, pregnant sheep in a farm environment with fecal samples consistently negative for nematode eggs, coccidian oocysts, and adequate diet (good pasture), did not develop anemia (HCT < 0.27), and the hematology (HCT and HGB) and protein concentrations (total protein and albumin) were not different in pregnant or non-pregnant ewes [34].

In the present study, erythrocytes parameters were significantly (P<0.01) determined by the reproductive stage, excepting MCV and MCHC, and the season (TABLE IV). For the red cell indices (MCV, MCH, MCHC) and platelet parameters (PLT), any significant difference was found between anemia groups (P>0.05) except one only marked increase in anemic ewes during the weaning period for MCH and MCHC at week 10 and PLT at week 8 p.p (FIG. 2F, FIG. 2G, and FIG. 2H, respectively). The effect of internal parasites on these parameters was expressed by the impact of the level of their egg shedding, which was significant for that of gastrointestinal nematodes (GIN), excepting on hemoglobin concentration, but not for that of Eimeria. For that of Fasciola, it was significant only for hemoglobin and the red cell indices (MCV, MCH, and MCHC). The interaction of the reproductive stage with this indicator of internal parasites burden severity had a significant effect on changes in most erythrocytes parameters (P<0.01) excluding that of interaction with Fasciola FEC level on hemoglobin and that of interaction with Eimeria oocyst count on MCH and MCHC (P>0.05).

For platelet count variation (TABLE IV), a significant effect of individual factors (anemia status and reproductive stage) and the shedding level of nematode eggs was revealed (P<0.01). In addition, it appeared to be influenced by the interaction of the breeding stage with the level of excretion of GIN eggs and Coccidia (P<0.01) but not with that of Fasciola (P>0.05). However, this parameter was not affected by the season or Coccidia and Fasciola FEC levels (P>0.05).

In a study conducted on West African Dwarf sheep reared under a semi-intensive system, no significant changes in HCT, RBC, HGB, MCV, MCH, or MCHC values during the postpartum period (between days 5 and 30 p.p.) were noted [35]. In agreement with the latter findings, MCV did not reveal a decrease in values, which goes hand in hand with the number of red blood cells during the present study. Thus,

these results are in contrast with those observed by Bezerra et al. [36] who reported a decrease in MCV during the different stages of reproduction with a recovery at weaning. However, in the present study, the interaction of the reproductive stage with the three types of parasites had a significant effect on MCV. A similar decrease in the recorded values of hematological parameters mainly HCT, HGB and RBC was noted in sheep naturally infected by Fasciola hepatica [37].

CONCLUSIONS

The present study highlighted significant variation in hematological parameters in anemic ewes in response to the combined effects of internal parasitism and the metabolic and endocrine changes involved in the transition from pregnancy to the postpartum period. Under the Mediterranean climate favorable to the development of internal parasites, infections during these critical reproductive periods remain an important health threat that should be considered and monitored by biological markers for better flock management. The study could not determine which species of internal parasites was the main agent compromising the hematological status of animals, therefore to select the most effective anthelmintic drug to be used in the control program. This seems essential from a research perspective to assess the potential resistance behavior of their infective agents in response to anthelmintic molecules under the influence of host-related factors.

Conflict of interests

The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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