J Parasit Dis (July-Sept 2021) 45(3):599–605
https://doi.org/10.1007/s12639-020-01336-z

ORIGINAL ARTICLE

Lamb's *Eimeria* infections raised in a steppic region and their impacts on clinical indicators (FAMACHA© and Disco)

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Received: 29 August 2020 / Accepted: 4 December 2020 / Published online: 6 January 2021

Abstract A total of 290 lambs in 29 small flocks were studied from October to January 2020, conducted in a semi-extensive rearing system. The objective was to identify by the microscopic morphology of oocysts the *Eimeria* species that infect lambs in a steppe region of eastern Algeria, as well as to evaluate on these parasites two clinical indicators of diarrhea (Disco) and anemia (FAMACHA©) used in targeted selective treatments against gastrointestinal nematodes (GIN). This present work completes the previous evaluations carried out in steppe conditions in our laboratory on GIN and cestodes. All these enteric parasites can influence these indicators. They constitute by their morbidity and their presence, alone or concomitantly, a factor determining or aggravating diarrhea, the main problem of sheep farming. The prevalence of *Eimeria* sp. oocysts was 56.2%. Eight species were identified: *E. intricata* (50.3%), *E. bakuensis* (43.6%), *E. ovinoidalis* (27.6%), *E. crandallis* (23.9%), *E. parva* (20.9%), *E. weybridgensis* (13.5%), *E. pallida* (9.2%) and *E. ashata* (6.7%). Unlike GIN and Cestodes where only Disco was affected, *E. ovinoidalis* was strongly affecting both indicators, while *E. crandalis* was slightly less affecting. This confirms the pathogenicity of these two species and suggests that their presence should also be taken into consideration.

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Keywords *Eimeria* spp. · Lamb · FAMACHA© · Disco · Steppe

Introduction

Lamb coccidiosis is due to the development in the intestines of 11 (Sattar and Şükran 2015; Alcala-Canto et al. 2020; El-Alfy et al. 2020) or 14 species of *Eimeria* sp. (Pellérdy 1974; Levine 1985; Platzer et al. 2005; Saratsis et al. 2011) specific strictly to sheep. Its impact is essentially economic, linked to visible sub-clinical infections (Lopes et al. 2013; Mohamaden et al. 2018), in particular in the context of intensive livestock farming systems with high animal density (Tomczuk et al. 2015). The latter promotes the exogenous maturation of the oocyst (sporogony), in 2–7 days depending on the *Eimeria* species under particular environmental conditions: moisture, oxygen, and temperature. Moreover, sporulated oocysts are highly resistant in the outdoor environment for several months or even more than a year (Chartier and Paraud 2012), facilitating infestation. Thus, host proliferation is very high and each oocyst ingested could be responsible for up to 30 million oocysts excreted in feces (Gregory and Catchpole 1987). Ingested oocysts release sporozoites that invade the epithelial cells of the small intestine and transform into schizonts. The schizonts begin their asexual multiplication (schizogony), releasing schizozoites that invade the epithelial cells of the large intestine where sexual multiplication occurs (gamogony) that will lead to the formation of non sporulated oocysts that will be released with the faeces into the external environment.

The economic impact may also be related to the direct clinical consequences of diarrhea, on animal growth and mortality, which may be as high as 15% (Craig 1986).

Diarrhea, which is generally more sporadic, is due to the destruction of intestinal epithelial cells and also leads to a massive change in the digestive microflora, which is a determining or aggravating factor in diarrhea (Mohammed et al. 2000). It is also related to the infectious dose of oocysts (Gregory et al. 1989a) and aggravated by concurrent bacterial, viral (Wright and Coop 2007) and gastrointestinal parasites (Taylor 2009). A recent impact is also to be considered with the description of Eimiridae resistance in goats to sulfonamides (Hsieh et al. 2020) and lamb to toltrazuril (Odden et al. 2018).

Clinical indicators such as diarrhea (Disco) (Cabaret et al. 2006) or anemia (FAMACHA©) (Van Wyk and Bath 2002), are used as a measure for targeted selective treatments against gastrointestinal nematodes (GIN). Owing to the spread of anthelmintic resistance in GIN, targeted selective treatments are being undertaken only for animals identified as sick to reduce the use of anthelmintics. Disco was found to be the most relevant indicator in the steppe regions of eastern Algeria and Morocco (Ouzir et al. 2011; Bentounsi et al. 2012). FAMACHA© scores and body weight have not been reliable indicators for detecting lambs in need of treatment in our conditions (Bentounsi et al. 2012). Also, in a second study in the steppe regions of eastern Algeria (Meradi et al. 2019), only Disco was associated with the cestode infestation observed at the following prevalences: *Moniezia expansa* (5.18%), *Avitellina centripunctata* (3.35%), *Stilesia globipunctata* (0.97%) and *Moniezia benedeni* (0.36%). Enteric coccidiosis may interfere with Disco or with the FAMACHA© score. Their clinical impact was not measured by these clinical indicators and could bias the selection of sheep to anthelmintic treatment against GIN. It was evaluated in this paper using clinical indicators of diarrhea (Disco) and anemia (FAMACHA©). The coccidian fauna in the Algerian steppe region is unknown, its description is the second objective of this work.

Materials and methods

Region and study lambs

This study was carried out in twenty-nine small farms (40–100 sheep on average per farm), located in a semi-arid region of North-eastern Algeria (Batna) from October to January 2020. A total of 10 lambs (Ouled Djellal breed) are randomly selected from each farm, were aged between 3 and 6 months. In usual farming practices in this study period, our experimental lambs were confined with adult sheep at night in a sheepfold and received roughage and concentrate in their diet. They are maintained at grazing during the day under a semi-intensive rearing system with often other herds of ruminants. Sometimes there are introductions of adult or young sheep purchased. These farms are often characterized by poor hygiene practices. No specific treatment against *Eimeria* was prescribed to the lambs.

Clinical indicators (FAMACHA© and Disco)

The FAMACHA© system is a tool that matches the color of the eye mucous membranes of small ruminants with a laminated color chart showing 5 color categories that correspond to different levels of anaemia: 1 = red, non-anaemic; 2 = red-pink, non-anaemic; 3 = pink, mildly anaemic; 4 = pink-white, anaemic; 5 = white, severely anaemic (Van Wyk and Bath 2002). The DISCO indicator classifies the consistency of the faeces according to 3 scores (Cabaret et al., 2006): 1 corresponds to normal sheep faeces in pellets, 2 is for "soft" faeces (similar to cow pat) and 3 is for diarrhoea (semi-liquid faeces). These clinical indicators and fresh faecal samples were collected from each experimental lambs.

Parasitological study

Fecal samples were taken from the rectum of lambs. Identified, they are transported in cold isothermal boxes to the laboratory and analyzed for the presence of *Eimeria* oocysts, cestodes and to estimate GIN.

The intensity of GIN infection was estimated by counting the number of eggs per gram of feces (EPG) with a modified McMaster method using a saline solution (1.18 density) sensitive to 15 EPG of feces. In the case where the McMaster method did not detect any eggs, a flotation method was also performed and was determined to be sensitive to 7.5 EPG (Raynaud 1970). The identification of the eggs was limited to the parasitic genus, such as *Nematodirus*, *Marshallagia* and other GIN.

For *Eimeria* infected lambs, a coproculture technique was carried out for each lamb to identify *Eimeria* species. Coproculture was undertaken to stimulate sporulation in the solution of 2.5% potassium dichromate ($K_2Cr_2O_7$) for 7 days at 26 °C. The criteria for the diagnosis of the oocyst are morphological characters of the unsporulated and sporulated oocysts include (the size, the shape, the color and texture of the oocyst wall, the presence or absence of a micropyle and polar cap, the presence or absence of oocystal and sporocystal residues and stieda bodies) (Eckert et al. 1995; Wang et al. 2010).

Statistical analyses

Ordinal logistic regression models were performed to assess the association between Disco, FAMACHA©, and

the following variables: Presence or absence of *Eimeria* infection, type of infection (single or mixed infection), *Eimeria* species and *Eimeria* species associations. The Odd Ratios which are the ratios between the risk factor of *Eimeria* infection or *Eimeria* species (explanatory variables) and the clinical indicators (dependent variables) were calculated. When OR = 1: explanatory variables don't affect the studied clinical indicators, when OR > 1 explanatory variables influence on indicators and are associated with higher scores of FAMACHA© and Disco (positive association). When OR < 1: the explanatory variables are associated with the lower scores of clinical indicators (negative association). 95% confidence intervals (CIs) were considered and a value of $p < 0.05$ was considered significant.

Additionally, an analysis of variance (ANOVA) was carried out, followed by a Tukey post hoc test to compare Disco and FAMACHA© of the different parasitological groups designed. A Spearman correlation (r_s) between clinical indicators and infection were also performed. All analyzes were carried out using SPSS 20 software.

Results

Prevalence and intensity of digestive-tract parasites

The prevalence of *Eimeria* oocysts was 56.2% (163/290). Eight *Eimeria* species were identified with the following prevalence rates: *E. intricata* (50.3%), *E. bakuensis* (43.6%), *E. ovinoidalis* (27.6%), *E. crandallis* (23.9%), *E. parva* (20.9%), *E. weybridgensis* (13.5%), *E. pallida* (9.2%) and *E. ashata* (6.7%).

The prevalence of *Moniezia expansa* was 31.7% and that GIN was 79.6%. Their EPG was low (Table 1).

Depending on the nature of the parasitism, three parasitological categories were observed in the study: *Eimeria* sp. infection only (33 lambs), Nematodes infection only (69 lambs), and Nematodes and *Moniezia expansa* infection (32 lambs) (Table 2). Single infection prevalence was 51.5% and 48.4% of infected lambs had mixed infections with at least two species.

Prevalence of clinical indicators

The FAMACHA© scores ranged from 1 to 5 and were found in 57.2%, 19.3%, 16.2%, 6.2% and 1.03% of the lambs, respectively. The Disco values (from 1 to 3) were 64.5%, 13.4%, and 22.06% of the lambs, respectively.

Impacts of parasitological categories on clinical indicators using ANOVA and Spearman correlations

By ANOVA, Disco and FAMACHA© were significantly ($p = 0.000$) higher in the *Eimeria* infection category only (Table 2). Disco ($rs = 0.50$; $p = 0.000$) and FAMACHA© ($rs = 0.46$; $p = 0.000$) were correlated with the category harbouring *Eimeria* infection only according to Spearman correlations.

Effects of presence/absence and of Eimeria infection on clinical indicators using the ordinal logistic regression

Lambs infected with *Eimeria* sp. (Table 3) have significantly high scores of Disco (OR = 1.75; $p = 0.000$) and FAMACHA © (OR = 2.21; $p = 0.000$). They have more Disco score 2 (OR = 2; $p = 0.000$), Disco score 3 (OR = 9.66; $p = 0.000$), and more FAMACHA© score 3 (OR = 5.71; $p = 0.000$) and FAMACHA© score 4 (OR = 17; $p = 0.000$).

Infection with a single species is more related to high scores for both indicators than mixed infections (Table 3). Lambs infested with a single species of *Eimeria* had higher Disco score 2 (OR = 2.25; $p = 0.000$), Disco score 3 (OR = 1.63; $p = 0.000$), and FAMACHA© score 3 (OR = 1.85; $p = 0.000$).

Impacts of *Eimeria* sp. species on clinical indicators using the ordinal logistic regression

Lambs infected with *E. ovinoidalis* had highly elevated Disco (OR = 5.81; $p = 0.000$) and FAMACHA© (OR = 5.98; $p = 0.000$) scores. Then, those infected by *E. crandallis*: Disco (OR = 2.58; $p = 0.000$) and FAMACHA© (OR = 1.88; $p = 0.000$) (Table 4).

Table 1 Prevalence of gastrointestinal nematodes of 290 lambs according to their faecal egg counts

Faecal egg count	Prevalence of gastrointestinal nematodes (%)		
	Marshallagia	*Nematodirus*	Other gastrointestinal nematodes
7–100	46.2	52.1	47.2
100–300	2.4	6.9	19.3
Over than 300	0.7	0.7	3.4

Table 2 Clinical indicators in relation to parasitological categories of lambs showing mean ± standard deviation (M ± SD)

Parasitological categories observed (no. of lambs)	Clinical indicators	
	FAMACHA© (M ± SD)	Disco (M ± SD)
Eimeria infection only (33)	2.33 ± 0.99	2.03 ± 0.95
Nematode infection only (69)	1.35 ± 0.63	1.25 ± 0.57
Nematode and *Moniezia expansa* infection (32)	1.41 ± 0.71	1.13 ± 0.42

no number, *M* mean, *SD* standard deviation

Table 3 Associations between presence/absence and *Eimeria* infection on clinical indicators determined using the ordinal logistic regression

	Eimeria infection		Type infection	
	Absence	Presence	Single	Mixed
Total number of lambs	127	163	84	79
Disco				
1	108	79	30	49
2	13	**26**	**18**	8
3	6	**58**	**36**	22
p value		**0.000**	**0.003**	
Odd ratio (95% CI)		1.75 (1.03–2.91)	2.48 (1.31–2.80)	
FAMACHA©				
1	90	76	31	45
2	29	27	16	11
3	7	**40**	**26**	14
4	1	**17**	8	9
5	0	3	3	0
p value		**0.000**	**0.019**	
Odd ratio (95% CI)		2.21 (1.09–4.49)	7.32 (5.11–8.26)	

Significant in bold

Lambs infected with *E. ovinoidalis* had higher Disco score 3 (OR = 2.02; $p = 0.000$), FAMACHA© score 3 (OR = 1.91; $p = 0.000$) and FAMACHA© score 4 (OR = 6.25; $p = 0.000$). Lambs infected by *E. crandallis* had higher Disco score 3 (OR = 1.36; $p = 0.000$), FAMACHA© score 3 (OR = 1.15; $p = 0.000$) and FAMACHA© score 4 (OR = 2.75; $p = 0.000$).

Of the associations observed, only lambs infected with *E. ovinoidalis* and *E. crandallis* had high scores for Disco (OR = 2.94; $p = 0.000$) and FAMACHA© (OR = 2.63; $p = 0.000$) (Table 5).

Discussion

In contrast to, subtropical and temperate regions, where prevalences are high in lambs, from 100% in Brazil (Lopes et al. 2013) and 93.1% in China (Wang et al. 2010). The overall prevalence of *Eimeria* infection observed in our study was moderate (56.2%). Similar prevalences in steppe regions of the world were reported: 44.5% in Algeria (Boulkaboul and Moulaye 2006), 56% in Morocco (Paliargues et al. 2007), 50.4% in Egypt (Mohamaden et al. 2018), 43.70% in Brazil (De Macedo et al. 2019), 50.7% in India (Swarnkar et al. 2010) and 50.74% in Mexico (Alcala-Canto et al. 2020). However, other research has also reported that prevalence may be high in semi-arid areas and coccidiosis is qualified as a pathology of sheep in these areas (Carrau et al. 2018). The differences observed may be related to the period studied. Our choice (October–January) was the most favorable, due to autumn rains and winter confinement, which are propitious to oocyst sporulation and resistance and to infestation, as observed in the annual dynamics (Tomczuk et al. 2015; Mohamaden et al. 2018; Sun et al. 2018). Also, the stress of confinement

Table 4 Associations between *Eimeria* sp. species and clinical indicators determined using the ordinal logistic regression

	E. ovinoidalis	*E. crandallis*	*E. intricata*	*E. pallida*	*E. ashata*	*E. weybridgensis*	*E. parva*	*E. bakuensis*
Total number of lambs (%)	45 (27.6)	39 (23.9)	82 (50.3)	15 (9.2)	11 (6.7)	22 (13.5)	34 (20.9)	71 (43.6)
Disco								
1	0	0	66	14	7	19	28	58
2	1	10	13	1	3	3	5	9
3	44	29	3	0	1	0	1	4
p value	**0.000**	**0.000**	**0.000**	**0.006**	0.181	**0.001**	**0.000**	**0.000**
Odd ratio (95% CI)	5.81 (3.75 to 7.87)	2.58 (1.74 to 3.43)	0.04 (− 4.12 to 2.55)	0.05 (− 5.01 to 0.84)	0.42 (− 2.15 to 0.48)	0.11 (− 3.54 to 0.95)	0.12 (− 3.04 to 1.11)	0.06 (− 3.57 to 2.05)
FAMACHA©								
1	0	0	64	14	8	18	27	52
2	0	14	12	1	3	3	5	12
3	26	15	6	0	0	1	2	7
4	16	10	0	0	0	0	0	0
5	3	0	0	0	0	0	0	0
p value	**0.000**	**0.000**	**0.000**	**0.021**	**0.05**	**0.000**	**0.000**	**0.000**
Odd ratio (95% CI)	5.98 (3.91 to 8.05)	1.88 (1.18 to 2.59)	0.04 (− 3.8 to 2.38)	0.08 (− 4.6 to 0.37)	0.24 (− 2.82 to 0.80)	0.14 (− 3.10 to 0.80)	0.14 (− 2.85 to 1.03)	0.11 (− 2.91 to 1.55)

Significant in bold

Table 5 Effects of *Eimeria* sp. associations observed on clinical indicators using the ordinal logistic regression

Associated *Eimeria* sp. observed	Number of lambs (%)	*p* value	
		Disco	FAMACHA©
E. ovinoidalis/			
E. crandallis	**19 (24)**	**0.000**	**0.000**
E. bakuensis/			
E. intricata	11 (13.9)	0.419	0.465
E. intricata + *E. ashata*	4 (5)	0.487	0.496
E. intricata + *E. weybridgensis*	7 (8.8)	0.185	0.581
E. intricata + *E. pallida*	2 (2.5)	0.160	0.871
E. intricata + *E. parva*	11 (13.9)	0.096	0.190
E. intricata + *E. ashata* + *E. Weybridgensis*	2 (2.5)	0.502	0.864
E. intricata + *E. parva* + *E. ashata*	5 (6.3)	0.628	0.987
E. intricata + *E. parva* + *E. Weybridgensis*	5 (6.3)	0.737	0.318
E. intricata + *E. parva* + *E. pallida* + *E. weybridgensis*	8 (10.1)	0.511	0.595
E. intricata/			
E. parva + *E. pallida*	5 (6.3)	0.349	0.397

Significant in bold

compromises the immune responses of animals housed in these conditions (Chartier and Paraud 2012; Carrau et al. 2018).

Among the eight species identified, the most prevalent were *E. intricata*, *E. bakuensis*, then *E. ovinoidalis*, *E. crandallis* and *E. parva*. There are no local comparative data on this parasitism. In other regions, these species were most common in Spain (Carrau et al. 2018), Italy (Scala et al. 2014) and temperate areas of western Europe (Reeg et al. 2005). In the steppe region of Brazil, *E. ovinoidalis*,

E. parva and *E. crandallis* were the most distributed (De Macedo et al. 2019). In the arid climate, *E. ahasta*, *E. crandalis* and *E. bakuensis* were the most prevalent (Mohamaden et al. 2018; Al-Saadoon and Al-Rubaie 2018). It is difficult to say that there is a particular geographical area of distribution for any of the coccidia species (Chartier and Paraud 2012). *E. ovinoidalis* was most widespread in very dry, dry and temperate climates in Mexico (Alcala-Canto et al. 2020). Infestation by a single species in half of the population studied remains a particular observation in this study. Mixed infection is generally more common (Kheirandish et al. 2014 and Mohamaden et al. 2018).

We observed that only *Eimeria* infection was associated with Disco and FAMACHA© scores. There was no impact on these indicators in lambs parasitized by GIN and *Moniezia expansa*.

We limited the age of the lambs studied to a minimum of 3 months to moderate the influence of other factors, such as neonatal disease (Jacobson et al. 2020), or susceptibility to infection, due to the lack of protective immunity in the youngest lambs which seems to settle after the peak excretion observed at weaning (Chartier and Paraud 2012). Immunity is early for mainly dominant species (Reeg et al. 2005).

Concerning the differential pathogenic action of *Eimeria* species. *E. ovinoidalis*, which affects epithelial cells of the large intestine, is qualified as the most pathogenic species, followed by *E. crandallis* and possibly *E. ahsata* (Gregory et al. 1989b). It is in this order that the impact on disco and FAMACHA© score was found in our results. The impact by *E. ovinoidalis* was much greater.

We limited ourselves to considering the clinical impact according to the coccidian prevalence. Taking into account the intensity of the infection in later studies would further clarify this aspect.

On the parasitic fauna of the gastrointestinal tract of the steppe regions evaluated in our laboratory, this is the first observation of a parasitic influence on FAMACHA©, recorded in this study by the *Eimeria*. These enteric parasites interfere directly with the disco or indirectly with FAMACHA© score. Moreover, the hemorrhagic that may accompany diarrhea, the main symptom of coccidiosis (Foreyt 1990), still interferes directly with FAMACHA©. This simultaneous influence of FAMACHA© and Disco score can be considered as a distinctive sign of this affection in lambs in steppe areas.

Disco was also in this study a good indicator of *Eimeria* infections as well as those due to GIN (Bentounsi et al. 2012) and cestodes (Meradi et al. 2019). Therefore, in our steppe conditions, it remains a more common clinical indicator and must be accompanied by a parasitological diagnosis for the proper management of gastrointestinal infections.

Acknowledgements The authors would like to express their special thanks to the students Chahrazed Benderradji and Lamia Nouioua for their collaboration in this work.

Author contributions Investigation, Methodology and Writing were performed by [SM]. Supervision, Writing and Reviewing were performed by [BB].

Funding The author(s) received no specific funding for this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All experimental methods were conducted in accordance with the ethical standards. The authors were permitted to collect the faeces samples and to manipulate the animals by their owners. No further authorization was required for the research and field studies.

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