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Genetic Selection for Dairy Traits and Resistance to Mastitis and Gastrointestinal Nematodes Has No Impact on *Cryptosporidium* spp. and *Eimeria* spp. Natural Infections in Blond-Faced Manech Lambs

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Simple Summary: Genetic selection, for both production and resistance traits, enables breeds to be improved to produce high-performance animals that are less susceptible to disease. However, breeders are concerned that animals selected are more susceptible to parasitis diseases, such as *Cryptosporidium* and *Eimeria* parasites, infecting young lambs and causing high morbidity and mortality in farm. To answer this question, we monitored Blond-Faced Manech breed ewe lambs from selected sire for milk production traits (yield, fat and protein contains), mastitis resistance (udder conformation and count cells in milk) and gastro-intestinal nematode resistance, by fecal examination to evaluate natural infection by these parasites. The results of this monitoring show that in this breed, selecting animals with more resistance to disease or better production does not make them more susceptible to *Cryptosporidium* and *Eimeria* parasites.

Abstract: (1) Background: Sheep have been selected to improve their milk production. Some local dairy breeds, such as the Blond-Faced Manech (BFM) in south-west France, also select for resistance to mastitis and gastro-intestinal nematodes. However, genetic selection can also deteriorate certain traits, to the benefit of improving others. Breeders are particularly concerned about infections by digestive protozoa of the *Cryptosporidium* and *Eimeria* genus, which infect lambs at an early age and can compromise their future dairy careers. (2) Methods: 159 ewe lambs form geneticaly selected sire for milk production trait, mastitis and gastroinestinal nematode resistance, were monitored in 11 BFM farms. Individual fecal samples at 15, 30, 60 and 120 days of age were performed to detect infections by *Cryptosporidium* spp. (PCR targeting 18S rRNA and sequencing) or *Eimeria* spp. (Fecal count and morphological examination of oocysts) with species identifications. All infections remained asymptomatic. The genetic selection of rams did not affect the infection of ewe lambs for *Cryptosporidium* or the intensities of oocyst excretion of *Eimeria* species in their offspring. (4) Conclusion: This result does not indicate that genetic selection leads to a higher susceptibility of ewe lambs to *Cryptosporidium* sp. and *Eimeria* sp. natural infection in BFM breed.

Keywords: sheep; *Cryptosporidium*; *Eimeria*; genetic selection

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1. Introduction

Genetic selection of animals is a way to improve farm animal breeds on different criteria of production, resistance or resilience. In France, the organization of the dairy sheep industry, using studs of rams and artificial insemination (AI) for pure-bred selection, enables rapid dissemination of genetic progress. As the primary selection criterion is milk production expressed by females, milk recording has been successfully implemented and estimated breeding values (EBV) have been calculated for breeding males [1]. In almost 50 years, ewe's milk production in France has almost quintupled [2]. On the strength of this efficient system, sheep breed selection organizations have decided to co-select on additional criteria, such as milk quality, mastitis indicators or, more recently, genetic resistance to gastrointestinal nematodes (GIN) [3] in some breed like Blond-Faced Manech (BFM), a dairy sheep breed from the southwestern of France. However, not all traits co-evolve in the same direction, and interactions are possible. For example, unfavorable genetic correlations between dairy traits (milk yield, fat and protein contents) and resistance to GIN have been estimated in the BFM population [4]; these correlations are weakly unfavorable, suggesting that genetic selection on milk production over the past has led to reduced resistance to GIN. However, given the relatively low magnitude of these correlations, this potential antagonism unlikely represents a solid limit to selection [4].

Besides trade-offs between production traits and health traits, trade-offs may also occur among health traits. GINs are not the only problematic parasites in sheep farming. It should also be checked that selecting individuals that are more resistant to GIN does not sensitize them to other parasites, such as *Cryptosporidium* sp. and *Eimeria* sp. Indeed, *Cryptosporidium* sp. and *Eimeria* sp. are considered to be highly pathogenic for newborns and young lambs, respectively. Oocysts of both parasites are excreted by the ewes, thereby significantly contributing to the contamination of the environment of newborns at the time of lambing [5]. The contamination can spread rapidly by fecal-oral transmission in a contaminated environment [6,7]. *Cryptosporidium* sp. infect newborn lambs from their first day of life and can lead to the appearance of clinical signs within two weeks of age, causing severe diarrhea with high mortality rates and economic losses associated with impaired growth [8]. The main species identified in lambs are *Cryptosporidium xiaoi*, *C. ubiquitum*, and *C. parvum*, the latter being more problematic because some subtypes are zoonotic [9]. *Eimeria* spp. also infects lambs soon after birth. However, the onset of clinical signs occurs later, usually between 1 and 3 months of age, with diarrhea that may be hemorrhagic, dehydration, malabsorption, and dieback [10]. *Eimeria* species are very specific to their host, and 11 species have been identified in sheep, including the most pathogenic: *Eimeria crandallis* and *Eimeria ovinoidalis* [11,12]. Even in subclinical infections, *Eimeria* sp. significantly decrease the animal's growth rate, causing significant economic losses to the farmer [13,14]. During their first months of life, lambs mount a specific immune response that is unsufficient to eliminate these parasites completely but which could permit their containment. It is well known that a Th1-based adaptive immune response is of critical for protection against intracellular pathogens such as *Cryptosporidium* sp. [15] and *Toxoplasma gondii* [16] in bovine and murine models. Adaptive immune responses in *Eimeria* infection in mammals are not well understood, but similar patterns of CD4+ Th1 polarization are presumably involved in the control of these infections by the host. In contrast, nematode parasites of the digestive tract, such as *Haemonchus contortus* elicit a Th2-polarized adaptive immune response in sheep [17–19]. Moreover, *H. contortus*-resistant breeds of sheep develop faster and higher Th2 immune responses than susceptible breeds [20]. Therefore, selection for resistance to *H. contortus* may lead to higher Th2 polarization immune responses. It was shown recently that natural resistance to worms enhanced bovine tuberculosis severity independently of active worm infection in wild African buffalo in co-infections [21], but no data is available in sheep.

Given the importance of protozoan infections in sheep, it is necessary to ensure that genetic selection for both production traits and disease resistance traits does not increase animals' susceptibility to *Cryptosporidium* spp. and *Eimeria* spp. during the first four months of lambs' life. Therefore, we compared the prevalence and intensities of *Cryptosporidium* spp. and *Eimeria* spp.

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infections in BFM lambs born form rams with different EBV for milk production (milk yield, protein and fat contains), mastitis resistance (cell count in milk) and GIN resistance (eggs excretion after infection) in eleven farms.

2. Materials and Methods

Farm and animal selection

The farms followed in this study were selected among voluntary BFM breeders, users of AI with rams that had EBV on the milk, mastitis, GIN resistance traits and a lambing period between November and January. All the farms were located in the French Basque Country, in the French Pyrénées-Atlantiques department, in southwestern France. A total of eleven farms were investigated in this study: five farms during the winter of 2017-2018 (Farms A to E) and six farms during the winter of 2018-2019 (Farms F to K) with 8 to 39 replacement ewe lambs monitored in each farm. The breeding practices were similar between farms, with the lambing period concentrated over 15 days, weaning of ewe lambs at approximately 40 days of age, followed by an anticoccidial treatment (generally with diclazuril, Vecoxan ND, MSD Santé Animale at 1 mg/kg BW). In farm C only, ewe lambs were separated from their mothers just after lambing and fed artificially. Throughout the study, the ewe lambs remained indoors. No clinical cases of cryptosporidiosis or coccidiosis were reported in the monitored farms. Over the two years of monitoring, a total of 195 ewe lambs from 42 different sires were analyzed. The ditribution of ewe lambs is shown in Table 1.

Table 1. Distribution of ewe lambs and number of rams used for artificial insemination by farm.

Year	Farm	Total monitored ewes lambs	Total rams with EBV used
2017-2018	A	17	8
	B	14	6
	C	18	10
	D	22	10
	E	10	6
2018-2019	F	21	6
	G	8	4
	H	17	4
	I	21	6
	J	39	14
	K	8	2

Sampling and laboratory analysis

The monitoring of parasitic infections during this study was based on microscopic and/or molecular analysis of fecal samples. Ewe lambs were sampled four times during the study: first time at 15 days old (D15), and then at 30 (D30), 60 (D60), and 120 (D120) days old. These sampling dates were chosen in order to obtain a measure of the critical age for *Cryptosporidium* infection (D15), a

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sampling before weaning and an eventually anticoccidial treatment (D30), a sampling far enough away from the weaning (D60), and a later point to account for the variation in the timing for the different *Eimeria* spp. (D120). The fecal samples were taken directly from the animal's rectum and were kept at +4 °C until analysis.

The presence of *Cryptosporidium* DNA in fecal samples at D15 was determined by duplex real-time PCR targeting 18S rRNA and LIB 13 genes [22]. All positive samples for *Cryptosporidium* sp. were genotyped in a second step by sequencing a fragment of the gp60 gene [23]. Species and subtypes of *Cryptosporidium* DNA were named using the established gp60 genotype nomenclature [24].

The following three fecal samples (D30, D60, and D120) were used to evaluate the intensities of Eimeria oocysts excretions and to identify *Eimeria* species in ewe lambs. They were analyzed individually using the modified McMaster technique [25]. Briefly, three grams of sample were weighed and suspended in 42 ml of saturated sodium chloride solution with a 1.2 g/mL density. After homogenization of the mixture, the solution was filtered three times through a tea strainer. While ensuring that the solution remained homogeneous after filtration, 1 ml was taken to fill both chambers of the McMaster slide. Total oocyst excretions were evaluated at 100x magnification. This total excretion was expressed in Oocysts Per Gram of feces (OPG). The sensitivity of the analysis was 50 OPG.

To identify *Eimeria* species, the fecal suspension was placed in a test tube to the brim and covered with a coverslip in contact with the suspension. A 15-minute flotation step was then performed to concentrate the *Eimeria* oocyst against the coverslip. The identification of Eimeria species between the slide and coverslip was done at 100x and 400x magnification and was based on morphological and morphometric criteria of the oocysts according to the guidelines of Eckert [26]. Oocyst measurements were performed with Zeiss image processing software Zen (Zen 2.6 blue edition, Carl Zeiss Microscopy GmbH, 2018). Species with very similar morphologies are classified in the same cluster. We thus obtain the following identification classes: *Eimeria pallida*/*Eimeria parva*; *Eimeria marsica*/*Eimeria ovinoidalis*, *Eimeria weybridgensis*/*Eimeria crandallis*, *Eimeria faurei*, *Eimeria granulosa*/*Eimeria bakuensis*, *Eimeria ahsata* and *Eimeria intricata*.

Data and statistical analysis

The descriptive analyses of the data were performed with R software version 4.1.1 (2024-06-14) [27] and R Studio version 1.4.1103 [28].

Different variables were used during statistical analysis and were described in Table 2. The methods for calculating milk and GIN relative EBV are presented by Aguerre and colleagues [4]. The EBV_milk initial values have been reduced by a factor 100 to match with the scale of other EBV. Mastitis resistance [29] and udder conformation [30] indexes were used too in statistical analysis.

Table 2. Descriptive data ewe lambs. EBV: Estimated Breeding Values. OPG: Oocysts Per Gram.

Qualitative Variables	Type	Description
Farm	Factor, 11 levels	1 level/farm monitored
Lambs	Factor, 195 levels	1 level/ewe lamb monitored
Litter_size	Factor, 3 levels	1 level : "1 lamb/litter"; n= 66 1 level : "2 lambs/litter"; n= 109 1 level : "3 or more lambs/litter" n= 20
Crypto	Factor, 2 levels	1 level: "positive": ewe lambs with *Cryptosporidium* PCR positive result; n= 56 1 level: "negative": ewe lambs with *Cryptosporidium* PCR negative result; n= 63

Quantitative Variables	Number of measures (missing information)	Description	Mean [Min; Max] (Standard deviation)
Age_D15	195	Age (in days) of ewe lambs at D15	15.26 [4;25] (4.5)

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OPGD30	151 (44)	*Eimeria* OPG measured at D30	11821 [0; 966 000] (86 934)
OPGD60	164 (31)	*Eimeria* OPG measured at D60	18842 [0; 294 000] (42 423)
OPGD120	155 (40)	*Eimeria* OPG measured at D120	9709 [0; 300 000] (27 987)
EBV_milk	195	EBV for milk yield trait from sire	1.253 [-5.1; 4.7] (1.99)
EBV_fat	195	EBV for fatty content in milk trait from sire	0.50 [-9.1; 9.5] (4.67)
EBV_protein	195	EBV for protein content in milk trait from sire	-0.3626 [-4; 7.3] (1.98)
EBV_cells	195	EBV for mastitis marker (number of cells in milk) trait from sire	-0.101 [-2.6; 1.8] (0.93)
EBV_udder	195	EBV for udder conformation trait from sire	0.02677 [-1.77; 1.98] (0.90)
EBV_GIN	195	EBV for GIN resistance trait from sire	-0.013 [-0.58; 0.71] (3.66)

We developed a binomial generalized linear model (GLM) to characterize the impact of EBV on *Cryptosporidium* spp. infections. Indeed, many ewe lambs presented a negative PCR result on these date. Thus, we analyzed them by classifying the results into two categories: presence or absence of the parasite. In this model, we tested the effects of the farm, the litter size, the age of the lamb at sampling, and the paternal EBV on milk (yield, fat and protein content), mastitis and GIN resistance. The significance of the effects was estimated using the Chi-squared test.

The model for *Cryptosporidium* infections was:

$$\text{Crypto} \sim \text{farm} + \text{litter\_size} + \text{Age\_D15} + \text{EBV\_milk} + \text{EBV\_fat} + \text{EBV\_protein} + \text{EBV\_cells} + \text{EBV\_udder} + \text{EBV\_GIN} \quad (1)$$

For *Eimeria* infection, we developed several generalized linear mixed model (GLMM) to assess the impact of EBV on these parasites. The first model was enabled us to compare the total OPG results obtained at D30, D60 and D120 with the influence of the age of the lamb, the litter size, the Cryptosporidium result at D15 and paternal EBV on milk (yield, fat and protein content), mastitis (udder conformation and cells in milk) and GIN resistance. Two variables with fixed effects were added to the model: the individual variable and the livestock variable. This addition corrects the effect of these two variables on the results. These models follow a negative binomial distribution, to which is added a correction for negative coprological analysis values. They were selected with the lowest values for Akaike's information criterion (AIC) and the lowest overdispersion ratio, compared with the complete model with the variables mentioned above. That is why in some models the variable « litter_size » was missing, because the model fitted better without it.

The complete model was therefore constructed as follows and corresponded to the models for total OPG :

$$\text{OPGtotal} \sim \text{Age} + \text{litter\_size} + \text{Crypto} + \text{EBV\_milk} + \text{EBV\_fat} + \text{EBV\_protein} + \text{EBV\_cells} + \text{EBV\_udder} + \text{EBV\_GIN} + 1|\text{Lambs} + 1|\text{Farm} \quad (2)$$

The other models are built in a very similar way, and focus on the quantity of oocysts of each species or clusters of species, excreted by lambs during the entire monitored period.

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For E. pallida/E. parva:

$$OPG\_a1a2 \sim Age + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (3)$$

For E. marsica/E. ovinoidalis:

$$OPG\_b3b4 \sim Age + litter\_size + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (5)$$

For E. faurei:

$$OPG\_b5 \sim Age + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (6)$$

For E. granulosa/E. bakuensis:

$$OPG\_b6b7 \sim Age + litter\_size + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (7)$$

For E. ahsata:

$$OPG\_b8 \sim Age + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (8)$$

For E. intricata:

$$OPG\_b9 \sim Age + litter\_size + Crypto + EBV\_milk + EBV\_fat + EBV\_protein + EBV\_cells + EBV\_udder + EBV\_GIN + 1|Lambs + 1|Farm \quad (9)$$

The significance of the effects was estimated using the Chi-squared test for all models.

3. Results

On D15, 120 ewe lambs were sampled and analyzed. Of these, 56 were positive for *Cryptosporidium* spp. in real-time PCR. Positive cases were present in almost all farms (nine out of the eleven studied farms: farms A, C, D, and E in 2017 and F, G, H, I, and K in 2018). Due to a logistical problem, samples from farm J could not be analyzed. Identification of the species and molecular genotyping was possible in six of the 56 positive animals (Table 3): three animals were infected with *Cryptosporidium xiaoi* (one ewe lamb at farms E, H, K), while the three others were infected with *Cryptosporidium parvum* (two ewe lambs at farm D and one ewe lamb at farm A).

Table 3. Results of Cryptosporidium DNA detection in ewe lambs by molecular analysis. ND: not determined due to sequencing failure.

Farm	Number of ewes lambs sampled	*Cryptosporidium* DNA positive sample	*Cryptosporidium* species and genotype
A	16	12	1 *C. parvum* (Cp IIdA24G1)

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B	10	0	-
C	14	11	ND
D	20	5	2 *C. parvum*
E	8	3	1 *Cryptosporidium xiaoi*
F	14	7	ND
G	7	3	ND
H	11	2	1 *C. xiaoi*
I	13	9	ND
J	-	-	-
K	6	4	1 *C. xiaoi*

The Cp IIdA24G1 *C. parvum* genotype was identified in this latter ewe lamb at farm A. GLM showed a significative impact of the farm (p= 0.000011) for *Cryptosporidium* infection. No other variable studied showed a significant effect, including the different paternal EBV, but it can be noted that older ewe lambs seem to be more often positive to infection than younger ones (p=0.07).

Natural *Eimeria* spp. infections were characterized at three sampling dates, namely D30, D60, and D120. All, except one of the ewe lambs, had a minimum of one positive examination for *Eimeria* oocyst excretion. On D30, a total of 151 ewe lamb samples were collected and analyzed, and 44 of these exhibited *Eimeria* spp. oocyst excretion (≥ 50 OPG). All farms except farm B had at least one ewe lamb that shed *Eimeria* oocysts at D30. Regarding the species in the 44 positive samples, *E. pallida*/*E. parva* were found in 20/44 samples, *E. marsica*/*E. ovinoidalis* in 20/44, *E. weybridgensis*/*E. crandallis* in 19/44, *E. faurei* in 2/44, *E. granulosa*/*E. bakuensis* in 10/44, *E. ahsata* in 9/44, and *E. intricata* in 1/44. The total oocyst excretion intensities ranged from 0 to 966 000 OPG (Table 2).

At D60, 164 ewe lamb samples were collected and analyzed, and 139 exhibited *Eimeria* oocyst excretion. The total oocyst excretion intensities ranged from 0 to 294 000 OPG, with an average of 18 842 OPG (Table 2). The abundances of the *Eimeria* species were as follows: *E. pallida*/*E. parva* in 97/139 samples, *E. marica*/*E. ovinoidalis* in 118/139, *E. weybridgensis*/*E. crandallis* in 91/139, *E. faurei* in 91/139, *E. granulosa*/*E. bakuensis* in 109/139, *E. ahsata* in 57/139, and *E. intricata* in 8/139.

At D120, 155 ewe lambs were sampled and analyzed and 151 of these were positive for *Eimeria* infection. The range of the shedding intensities was 0 to 300 000 OPG, with an average of 9 709 OPG (Table 2). The proportions of species found in the samples were as follows: *E. pallida*/*E. parva* in 110/151 samples, *E. marsica*/*E. ovinoidalis* in 144/151, *E. weybridgensis*/*E. crandallis* in 111/151, *E. faurei* in 81/151, *E. granulosa*/*E. bakuensis* in 139/151, *E. ahsata* in 112/151, and *E. intricata* in 39/151.

GLMM for the intensity of oocyst excretion showed an AIC of 4,285 and a overdispersion ratio of 15.7 (Table 4). None of the variables tested showed a significant impact, with all p-values between 0.12 and 0.81. For the intensity of oocyst excretion for each species, AICs ranged from 716 to 3 321 depending on the model, with overdispersion ratios ranging from 2.5 to 12 (Table 4). Very few significant effects were found in the models. No EBV had a significant impact on *Eimeria* oocyst excretions, either for total excretions or for species.

Table 4. Summary of GLMM characteristics for ovine *Eimeria*.

Model	Akaike's information criterion (AIC)	Overdispersion ratio	Significative effect
Total oocysts	4289.9	15.7	-
E. pallida/*E. parva*	2632.3	9.7	-
E. marsica/*E. ovinoidalis*	3321.3	12	-
E. weybridgensis/ *E. crandallis*	2518.9	9	-
E. faurei	2375.8	8.7	Age p = 0.003
E. granulosa/*E. bakuensis*	2873.4	10.6	-

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E. ahsata	1921	7	-
E. intricata	716.6	2.5	Litter_size p= 0.0069

For *E. intricata*, litter size significantly influences the amount of oocyst excretion in ewe lambs (p=0.0069), with greater excretion in ewe lambs without siblings. There is also less influence from udder conformation (p=0.07). For *E. faurei*, age has a significant impact (p=0.003) on oocyst excretion intensities: older lambs excrete more than younger ones. Ewe lambs from mastitis-resistant sires, with low number of cells in milk have a tendency (p= 0.066) to excrete more *E. faurei* oocysts. Finally, for species *E. pallida*/*E. parva* and *E. weybridgensis*/*E. crandallis*, ewe lambs from sires with the best EBV on udder-typpe tend to have higher oocyst excretion intensities on these species. For all other species, no significant impact or trend was found (respectively p=0.068 and p=0.070).

4. Discussion

We evaluated in the present study the impacts of genetic selection for production traits and resistance to mastitis and GIN on the resistance/susceptibility to *Cryptosporidium* spp. and *Eimeria* spp. parasites of the digestive tube, which are very common in lambs. The most striking result of this study is that current genetic selections did not affect the prevalences of *Cryptosporidium* and *Eimeria* infection and intensities of these parasites nor the relative abundances of the various species in the *Eimeria* genus in BFM breed in asymptomatic infections.

In this study, all the animals sampled did not express clinical symptoms of cryptosporidiosis, although the parasite's DNA was detected in nearly 47% of the ewe lambs sampled at D15. Unfortunately, as explained in Bordes et al. [31], the analysis of *Cryptosporidium* species infecting ewe lambs was limited due to mixed infections with different *Cryptosporidium* species. Indeed, the very low excretion intensities of *Cryptosporidium* oocysts, associated with co-infections of different species in the same ewe lambs, did not allow us to determine the species and subtypes in the vast majority of cases due to technical limitations. However, six samples could be characterized to this level of identification. *C. xiaoi*, common in sheep in Europe [32–34], and *C. parvum*, associated with the Cp IIdA24G1 genotype, were found. The Cp IIdA24G1 genotype of *C. parvum*, determined by sequencing of the *gp60* gene, was already widely known to infect lambs and goats kids [33,35] and has also been the cause of a human cryptosporidiosis outbreak [36]. Thus, there is a high degree of asymptomatic carriage of *Cryptosporidium* species in the investigated farms on ewe lambs, with have potentially zoonotic *C. parvum*. However, there was no effect of the paternal EBV on this carriage, suggesting no impact of current genetic selection of sires on *Cryptosporidium* infections in their ewe lambs. As in studies performed in cattle [37,38], only the farm of origin was found to be significant in promoting *Cryptosporidium* infection.

In the present study, the percentage of ewe lambs excreting *Eimeria* oocysts at D30 was low (30%), which is unusual, considering that ingestion of infective oocysts present in the lamb's environment frequently occurs quickly after birth [39]. In many studies, *Eimeria* oocyst excretions are commonly recovered in lambs as soon as 20 days of age [10,40], with a peak of excretion at approximately 30 to 40 days of age [14,41] followed by a decrease as a consequence of the lamb's immunity [14,41,42]. In our study, this peak was slightly shifted, as the highest intensities of excretion were recorded at D60, followed by the expected decrease at D120. It is difficult to explain the shift in excretion peak. The most probable reason is a low degree of contamination of *Eimeria* spp. in the BFM sheepfolds. In the breeding system of BFM dairy sheep farms, there is only one lambing period per year, as milk and cheese production are highly seasonal activities. Usually, sheepfolds are thoroughly cleaned between two lambing seasons. Moreover, we monitored *Cryptosporidium* and *Eimeria* infections in ewe lambs born from artificial inseminations, which are the very first lambs in the lambing season. This may thus explain why the oocyst contamination of the environment was low when the study started. Even though the excretion peak was at D60, a very high proportion of ewe lambs excreted *Eimeria* oocysts at D120 (97.4%). These results suggest that although the initial contamination with *Eimeria* oocysts was low in the farms, rapid contamination occurred in the ewe

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lambs throughout their first months of life. This is a strong point for estimating the link between genetic selection for enhanced resistance to GIN and *Eimeria* susceptibility, as all ewe lambs were exposed to *Eimeria* infection. In natural infections on farm, we cannot assess whether all of the ewe lambs are exposed to the same parasite pressure as is the case for experimental infections, in which similar infections in terms of species composition and the number of ingested oocysts could be performed. The proportions of ewe lambs excreting oocysts and the mean oocyst excretion intensities varied between the farms and the sampling dates, although within a farm, these parameters were similar in ewe lambs.

Regarding *Eimeria* species, high abundances of *E. pallida*/*E. parva*, *E. marsica*/*E. ovinoidalis*, and *E. weybridgensis*/*E. crandallis* were noted on the three sampling dates, to which *E. faurei* and *E. granulosa*/*E. bakuensis* were added at D60 and D120. This result is not surprising as these species are found in high proportions in European lambs [14,43]. The animal's age has a particular influence on the dynamics of *Eimeria* species. Indeed, at D120, older lambs excreted fewer *E. marsica*/*E. ovinoidalis* oocysts and more *E. granulosa*/*E. bakuensis* oocysts. This phenomenon is likely due to immunity of the lambs to the first species, which then allows the other species to become dominant [14,44,45].

No effect of the paternal EBV was found on the intensity of excretion of *Eimeria* species excreted by the ewe lambs, especialy for pathogenic species. Genetic selection of animals with better dairy performance and resistance to mastitis and gastrointestinal strongyles does not make ewe lambs more susceptible to the digestive protozoa *Cryptosporidium* spp. and *Eimeria* spp. On the other hand, they have no ameliorative effect. This selection can therefore be continued without any deleterious effect on these infections when the animals are young.

5. Conclusions

In this study, the influence of the genetic selection for production traits and resistance to mastitis and GIN in Blond-faced Manech rams was evaluated on natural *Cryptosporidium* spp. and *Eimeria* spp. infections in their offspring. Infections by these protozoan parasites were monitored in ewe lambs at four sampling dates (D15, D30, D60, and D120) by molecular analysis (for *Cryptosporidium* spp. infections) and microscopy (for *Eimeria* spp. infections). The overall *Cryptosporidium* spp. prevalence in ewe lambs was 50%, and *C. xiaoi* and *C. parvum* (including zoonotic subtypes), were identified. There was no influence of the genetic background of the sire on *Cryptosporidium* spp. oocyst shedding of their offspring. Similarly, we did not detect any influence of paternal EBV on the intensity of total *Eimeria* oocyst excretions and the pathogenic species such as *Eimeria ovinoidalis* and *Eimeria crandallis*. In conclusion, the current genetic selection of BFM sheep appears possible without rendering them more susceptible to *Cryptosporidium* spp. or *Eimeria* spp. infections during their young age.

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Abbreviations

The following abbreviations are used in this manuscript: AI : artificial insemination. AIC: Akaike's information criterion. BFM: Blond-Faced Manech. EBV: Estimated Breeding Values. GIN: GastroIntestinal Nematode. GLM: generalized linear model. GLMM: generalized linear mixed model. OPG: Oocysts Per Gram.

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