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Original Article

Fecal Egg Count and FAMACHA Score in Ewes

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ABSTRACT

Parasitic nematodes present a major cause of economic loss that impacts the sheep industry worldwide. The collection of scientific data from different regions and the introduction of reliable diagnostic methods can provide the instruments for control especially now when the situation is complicated by increased prevalence and development of anthelmintic resistance. This study aims to pay attention to fecal egg count (FEC) of ewes from the Southern part of Bulgaria and try to correlate results to FAMACHA scoring in the context of naturally occurring mixed-type gastrointestinal nematode infection.

Keywords: Gastrointestinal nematodes, Sheep, FEC, FAMACHA

INTRODUCTION

The sheep industry in Bulgaria comprises predominantly small-scaled farms keeping on average less than 500 to 1000 animals that are raised under grazing conditions during most of the year. According to data from the Ministry of agriculture, food, and forestry the total number of sheep in 2019 was equal to 1 280 983 (Bulgarian Ministry of Agriculture, Food and Forests, 2020). Herds are often mixed breeds with no pedigree register. Husbandry conditions can be described as poor to average. Deworming is usually done twice a year as a mass event and is rarely based on parasitological examination. The climate in the country is temperate, however, the weather in the Southern parts is mild and warm even during the winter. There are a

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few scientific studies on the current parasitological situation in Bulgaria (Radev *et al*., 2012; Iliev, 2019).

Parasitic diseases and gastrointestinal nematodes in the particular present among the main causes of production loss in sheep farming that impact negatively profitability (Roeber *et al*., 2013b). Consequences include a decrease in daily milk yield (Alberti *et al*., 2012) and a reduction in weight gain (Ilangopathy *et al*., 2019) due to inefficient food conversion (Jas *et al*., 2017) as well as a fall in wool production (Liu *et al*., 2003; Southcott *et al*., 2006).

One of the most important and widely used traits to estimate the severity of parasite burden is the fecal egg count (FEC). The method is inexpensive and easy to perform (Roeber *et al*., 2013a), though time-consuming, especially in larger herds. It has become the golden standard for diagnostics and follow-up and has been recommended by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.).

FEC should be checked with attention during the period of immune relaxation around parturition known as the periparturient rise (Gibbs, 1986; Chaney, 2012). This period is characterized by a significant increase in the number of parasite eggs that presents a major source of pasture contamination for naïve progeny (Sebastiano *et al*., 2017). Another important factor is the reactivation of hypobiotic larvae in spring (Abbott *et al*., 2012) and high fecal egg output usually one month after introduction to pasture (El-Ashram *et al*., 2017).

The need for a quick diagnostic strategy has led to the development of the FAMACHA colour chart- a scoring system from South Africa introduced to estimate the clinical manifestation of hemonchosis (van Wyk and Bath, 2002). It is based on anemia level and its relation to hematocrit results; the scoring is done against a standardized set of five colours ranging from red-pink (normal) to white (terminal anemia) (Malan *et al*., 2001).

This study aims to analyze FEC of ewes from a typical farming system in Southern Bulgaria and attempt to correlate results to FAMACHA scoring as well as identify the genus and species prevalence.

MATERIALS AND METHODS

1) Experimental Animals

The study was conducted from February to May 2021 in a flock of 800 sheep from dairy breeds, predominantly 4^{th} to 5^{th} generation Assaf crosses with local breeds. The experimental animals included 25 ewes during the lactation period. All were examined and found to be clinically healthy before the initiation of the test period.

2) Test Procedure

Biological materials were obtained and data were recorded in individual protocols. Blood for hematology was taken by jugular puncture in pre-labeled tubes. Feces were collected rectally and examined within 24 hours.

The study can be divided into three parts. It started end of February 2021 when ewes had given birth and were in the 6-8th week postpartum. The timing was chosen so it can coincide with the expected rise in FEC during the periparturient period. The follow-up was carried out at the beginning of April and then at the beginning of May when animals were already actively grazing.

Deworming was performed in August 2020 with levamisole/oxiclosanide oral suspension and later in December 2020 with ivermectin subcutaneously. Antihelmintics` type and regimen were chosen by the farmer as the herd is privately owned. No treatment was performed during the test period.

Due to the warm weather, the pasture season began early in March.

3) Parasitological Methods

Parasitology was carried out according to the modified McMaster technique using original McMaster slides (EGGZAMIN™) as described by Zajac and Conboy (2012). Fecal egg count is expressed as the number of eggs per gram of feces. It is an important variable in sheep for the prevention of parasite disease and the maintenance of efficient production.

Gastrointestinal strongyle identification on the level of genus was based on morphological data of nematode third-stage larvae isolated by the Modified Baermann's test from faecal culture after performing conventional larval cultivation (Zajac and Conboy, 2012; Kanchev *et al*., 2016).

4) FAMACHA Scoring System

FAMACHA scoring was done with the original FAMACHA colour chart developed by Malan *et al*. (2001) and introduced by van Wyk and Bath (2002). It is based on anemia level and its relation to hematocrit results. The color of the eye mucous membranes is matched to a chart of five categories with each corresponding to a definite hematocrit range. The scoring is done against a standardized set of five colours ranging from red-pink (normal) to white (terminal anemia) (Malan *et al*., 2001).

5) Hematology

The Mindray hematology analyzer BC-2800 Vet Automatic was used.

6) Statistical Analysis

Statistical analysis was conducted by Microsoft Excel 2016. Mean values and standard deviation (X±SD), correlation coefficient by the CORREL function, and significance value ($p < 0.05$) are represented in the following section.

RESULTS

1) Parasitological Results

FEC of ewes during the periparturient period (February 2021) was characterized by mean values of 688 ±554.78 EPG. From all 25 animals, there was 1 with FEC 50 EPG (№ 13); 4 with FEC 100 to 200 EPG (№ 3, 4, 12, 24), 11 with FEC 250 to 750 EPG (№ 6, 7, 8, 11, 14, 15, 18, 21, 22, 23, 25) and 9 with FEC above 750 EPG (№ 1, 2, 5, 9, 10, 16, 17, 19, 20). Therefore, the percentage distribution was 4% with EPG 50, 16% with EPG 100-200, 44% with EPG 250-750, and 36% with EPG above 750.

The examination in April 2021 showed mean values of 589.58 ±677.46 EPG. The individual distribution was 1 animal with EPG 0 (№ 13); 2 with EPG 50 (№ 12, 18); 6 with FEC from 100 to 200 EPG (№ 1, 2, 3, 4, 7); 11 with EPG 250-750 (№ 6, 8, 11, 14, 15, 16, 17, 21, 22, 23, 25); 5 with EPG above 750 (№ 5, 9, 10, 19, 20). Or there was 4% with EPG 0, 8% with EPG 50, 24% with EPG 100-200, 44% with EPG 250-750, and 20% with EPG above 750.

The follow-up in May resulted in a mean FEC of 447.92 ±628.9 EPG. There was 1 ewe with EPG 0 (№ 11); 7 with EPG 50 (№ 1, 7, 12, 13, 18, 23, 24); 5 with FEC 100-200 EPG (№ 2, 3, 4, 8, 14); 8 with EPG 250-750 (№ 5, 6, 15, 17, 19, 21, 22, 25); 3 with EPG above 750 (№ 9, 10, 20). Or there was 4% with EPG 0, 28% with EPG 50, 20% with EPG 100-200, 32% with EPG 250-750, and 12% with EPG above 750.

Helminthological study revealed the infestation with two members of *Trichostrongylidae* family belonging to genera *Trichostrongylus* and *Ostertagia* based on nematode third-stage larvae morphology. Ewes № 5, 9, 17 were also positive for nematodirosis based on egg identification.

P value is calculated: $p = 0.004$ for February/April, $p = 0.015$ for April/May, and $p = 0.0005$ for February/May. Results from FEC are represented in Table 1.

2) FAMACHA Scoring System and Hematology

FAMACHA scoring was carried out at the beginning and end of the test period. The mean values were 3.2 ± 0.4 in February and 3.12 ± 0.32 in May 2021. Most of the animals scored 3 according to the original colour chart. Only a few were in category 4. FAMACHA scoring is represented in Table 2. Results from hematology are represented in Table 3 and 4.

Table 1: Results from FEC (EPG) in February, April, and May 2021

FEC (EPG)	February 2021	April 2021	May 2021	Mean individual result
MEAN	688	589.58	447.92	
± SD	554.78	677.46	628.9	
MIN/MAX	50 / 2450	0 / 2800	0 / 2350	
1	900	100	50	350
2	800	100	150	350
3	150	200	200	183.3
4	100	200	150	150
5	800	800	750	783.3
6	300	450	250	333.3
7	300	150	50	166.7
8	750	400	200	450
9	2450	2200	2350	2333.3
10	1200	1450	1200	1283.3
11	450	350	0	266.7
12	200	50	50	100
13	50	0	50	33.3
14	300	300	200	266.7
15	350	300	300	316.6
16	1350	350	100	600
17	1350	750	450	850
18	350	50	50	150
19	1400	1300	750	1150
20	1200	2800	2300	2100
21	250	300	300	283.3
22	500	450	450	466.7
23	650	500	50	400
24	150	150	50	116.7
25	400	450	300	383.3

Table 2: FAMACHA score in February and May 2021.

FAMACHA score	February 2021	May 2021
MEAN	3.2	3.12
± SD	0.4	0.32
MIN/MAX	3 / 4	3 / 4
1	3	3
2	3	3
3	3	3
4	3	3
5	3	3
6	4	3
7	3	3
8	3	3
9	4	4
10	3	3
11	3	3
12	3	3
13	3	3
14	4	3
15	3	3
16	3	3
17	3	3
18	3	3
19	4	4
20	4	4
21	3	3
22	3	3
23	3	3
24	3	3
25	3	3

Table 3: Hematology results in February 2021

	WBC 10^9/L	RBC 10^{12}/L	HGB g/L	HCT %	MCV fL	MCH Pg	MCHC g/dL	PLT 10^9/L
MEAN	10.86	8.91	99.25	30.58	34.5	11.12	334.5	478.23
± SD	1.63	0.78	5.7	2.83	1.92	0.57	15.28	121.08
1	11.3	9.74	107	33.7	34.6	L 10.9	317	526
2	10.5	8.39	L 89	28.1	33.6	10.6	L 316	677
3	13.3	8.94	108	34.3	H 38.4	12	L 314	301
4	12.8	8.23	92	28.6	34.8	11.1	321	416
5	10.6	8.04	93	28.1	35	11.5	330	594
6	10.8	9.36	105	29.7	32.3	10.9	345	631
7	11	8.5	101	31.5	37.1	11.8	320	547
8	12.4	9.21	106	33.8	36.7	11.5	313	459
9	12.6	8.33	92	26.8	32.2	11	343	484
10	12.7	10.22	104	30.8	30.2	10.1	337	L 123
11	12.4	8.05	L 89	28.5	35.5	11	L 312	H 840
12	H 14.9	8.37	L 87	27.2	32.6	10.3	L 319	L 100
13	8.8	7.81	96	30.6	H 39.3	12.2	L 313	427
14	10.4	L 7.43	L 88	26.9	36.3	11.8	327	487
15	8.6	8.19	97	31	37.9	11.8	L 312	349
16	7.4	9.02	95	29.1	32.3	10.5	326	497
17	10.6	10.12	93	35	32.6	11.2	347	473
18	11.4	9.63	104	32.7	34	10.7	L 318	360
19	8.9	8.3	91	28	33.8	10.9	325	813
20	8.6	L 7.5	L 89	L 25.1	33.5	11.8	354	540
21	10.9	10.14	102	26.5	36.2	11	328	437
22	10.7	8.28	97	29.7	34.7	10.6	362	458
23	12.6	8.47	96	35.2	34.1	10.3	316	356
24	8.6	9.32	108	33.4	37.3	11.4	348	321
25	12.8	10.35	98	34.7	36.1	10.8	362	368

Table 4: Hematology results in May 2021

	WBC 10^9/L	RBC 10^{12}/L	HGB g/L	HCT %	MCV fL	MCH Pg	MCHC g/dL	PLT 10^9/L
MEAN	9.58	8.9	99.25	30.59	34.5	11.14	336.65	463.19
± SD	1.78	0.69	5.39	2.35	2.04	0.62	14.54	159.56
1	9.8	9.24	103	31.6	34.3	11.1	325	614
2	9.6	7.97	L 82	26.3	33	10.2	L 311	389
3	H 16.2	7.93	92	29.1	36.8	11.6	L 316	210
4	8.3	9.44	106	32.5	34.5	11.2	326	328
5	8.8	10.79	109	31.8	29.5	10.1	342	515
6	8.9	8.23	98	30	36.5	11.9	326	H 728
7	12.4	8.65	104	32	36.4	11.6	361	620
8	12.6	9.23	106	32.9	35.7	11.4	322	422
9	9.2	8.49	105	33	36.8	11.8	337	547
10	8.5	9.44	93	36	34.7	11.6	328	275
11	8.9	8.3	91	29.2	35.2	10.9	L 311	558
12	H 17.1	8.84	92	28.1	31.9	10.4	327	L 132
13	H 14.3	L 7.46	L 88	28.2	37.9	11.7	L 312	245
14	9.3	9.4	L 89	34	35.6	11.9	371	614
15	9.6	8.34	98	31	37.2	11.7	L 316	180
16	9.5	9.3	97	29.7	32	10.4	326	645
17	10.6	8.64	99	30.3	35.1	11.4	326	603
18	H 14.5	9.81	104	32	32.7	10.6	325	L 105
19	6	L 7.34	L 80	L 25.4	34.7	10.8	L 314	651
20	13.6	7.91	91	L 25.9	32.8	11.5	351	291
21	H 14.1	8.2	100	29.4	35.9	12.1	340	L 135
22	11.1	8.59	L 87	26.3	30.7	10.1	330	292
23	6.8	9.2	96	28.3	34.9	10.3	354	641
24	8.3	9.46	101	29.6	33.8	10.8	358	573
25	9.7	9.23	100	32.3	34.1	11.4	327	514

Ewes that fall in category 3 are assumed to be normal as they also show hematological results within the reference range. Individuals that scored 4 or had pale/anemic mucus membranes included № 6, 9, 14, 19, 20 in February and № 9, 19, 20 in May. It is observed that № 9, 19, and 20 repeats, while the score of № 6 and 14 has become 3.

As long as the FAMACHA system was developed for visual estimation of anemia, results should be compared with data from hematology. Ewes № 6 and 9 show no changes in CBC. Ewe № 14 is characterized by a decrease in RBC, hemoglobin, PCV in February, but only hemoglobin remains insignificantly low in May. Changes in RBC, hemoglobin, PCV, MCHC are seen in ewe № 19 in May. Ewe № 20 shows low RBC, hemoglobin, PCV in February, and hemoglobin in May.

The correlation coefficient between FEC and FAMACHA equals 0.416 in February and 0.813 in May.

DISCUSSION

The analysis of results shows that the percentage of animals with medium to high FEC (EPG 250-750) was 44% in February and April but decreased to 32% at the end of the study. Individuals with high FEC (EPG above 750) account for 36% during the periparturient period and decrease to 20% in April and 12% in May. The percentage of animals with low FEC (EPG 50) is characterized by increasing values: from 4% in February to 8% in April and 28% in May; from April on there are 4% with EPG 0.

The difference between test results is statistically relevant as manifested by the calculated p-value. This is better pronounced between February/April ($p = 0.004$) and February/May ($p = 0.0005$). The value is less significant when comparing April/May ($p = 0.015$), as test results become more evenly distributed after the end of the critical periparturient period.

Changes in mean values are also relevant. Mean FEC decreased from 688 ± 554.78 EPG in February to 589.58 ± 677.46 EPG in April and 447.92 ± 628.9 EPG in May though it could be expected that FEC should rise with the beginning of the pasture season and the spring warming. It should be noted again that the number of animals with high FEC decreased, while those with low FEC increased. This can be explained by the recovery of the immune response after the end of the critical periparturient period (Abbott, 2018). It is speculated that the rise in FEC can be due to low levels of antibodies in the gastrointestinal tract which allows the attachment of larvae and/or the activation of parasites from the state of hypobiosis (Jeffcoate *et al.*, 1992). After hypobiosis adult worms are capable of producing a large number of eggs (Gibbs, 1986), which can be the reason for the high percentage of ewes with FEC equal to or above 750 EPG.

The ability of an individual to limit or reduce the parasite load represents its natural resistance. This concept is widely discussed as a part of the sustainable parasite control approach (Hoste and Torres-Acosta, 2011). Therefore, it is worth dividing animals from this experiment into several groups.

The first group includes animals that show constantly low FEC (below 250 EPG). These are ewes № 3, 4, 12, 13, 24. This category can include ewes that are characterized by low FEC after the initial increase during the periparturient rise like ewe № 7.

Ewes with high FEC (above 750 EPG) include № 5, 9, 10, 19, 20. It is important to note that ewes № 5, 9, and 17 were also positive for nematodirosis.

The other animals can be categorized as having medium FEC (250-750 EPG). However, certain animals cannot be classified so straightforwardly. For example, ewes № 1, 2, and 16 with high FEC during the periparturient rise but significantly low in April and May (50-150 EPG). Ewe № 17 may also be included though the decrease in FEC is not as prominent.

The parasite invasion is controlled by mechanisms that suppress the growth, establishment, and survival of nematodes; however, the immune response to species that are localized in the abomasum is slower (McRae *et al*., 2015). The competence of the immune reactivity ensures the expulsion of adult worms and reduced fecundity of females (Kemper *et al*., 2010; Hendawy, 2018). An interesting phenomenon is the so-called "self-cure effect" when larvae and adult parasites are removed from the organism without the intake of anthelmintics (Garza, 2014). The expulsion relies on mucin hypersecretion (Benavides *et al*., 2016), high levels of histamine and leucotrienes as well as increased peristalsis (Alba-Hurtado and Muñoz-Guzmán, 2012).

It can be speculated that animals from the last group developed a "self-cure" reaction which resulted in the decrease in FEC. As it was mentioned ewes were regularly dewormed, but not during the experimental period. Nevertheless, results from FEC were predominantly medium to high in the February testing probably due to the consequences of the periparturient rise. However, many animals were able to withstand or overcome the infection which is demonstrated by the overall decrease in EPG.

The division into groups of low/medium/high FEC is useful to direct treatment decisions on the individual and herd levels. This method can also be applied as a selection tool to estimate the level of resistance/tolerance of sheep to gastrointestinal nematodes (Gauly and Erhardt, 2001; Vanimisetti *et al*., 2004; Bishop, 2009; Greeff *et al*., 2009; Bishop, 2012; Valilou *et al*., 2015). FEC is an important variable with known intermediate inheritance (Smith *et al*., 1999) that can influence the distribution of parasites within the population. It can be measured regularly to estimate the adverse effects of parasitism. Set as a selective trait in reproductive animals or as an indicator of infection severity, it can be used for decision-making about the selective treatment of the herd or the individual animal. The application of deworming to the whole population once or twice a year is no more recommended due to the possibility of parasite resistance and the wider concept of the benefits of refugia (van Wyk, 2001; van Wyk *et al*., 2006).

The finding of Trichostrongylus and Ostertagia nematodes indicate the possible wide spread of trichostrongylides among the population of sheep on pasture housing technology in Bulgaria which has been discussed in other regional studies as well (Radev *et al*., 2012; Iliev, 2019).

According to many authors FAMACHA score can be well correlated to a decrease in PCV in gastrointestinal nematode infections (Kaplan *et al*., 2004; Burke *et al*., 2007; Assefa, 2015). However, results from the current experiment showed that the method is not reliable enough to predict the presence of anemia. It should be mentioned that the system is specifically directed to the clinical consequences of hemonchosis. The infection in the Bulgarian context is expected to be mixed and there can be other blood-sucking species present (Di Loria *et al*., 2008; Radev *et al*., 2012; Iliev, 2019). The manifestations can also be influenced by the ratio between different parasites (Douch *et al*., 1996). Therefore, FAMACHA should not be used independently but can supplement the clinical examination. Information about the application of the colour chart in Europe is still insufficient but the conclusions published by Di Loria *et al*. (2008) are in accordance.

The correlation between FEC and FAMACHA is also widely discussed. Studies suggest that a higher FAMACHA score corresponds to a higher FEC (Notter *et al*., 2017; Galyon *et al*., 2020). However, the increase in FEC is variable (Cintra *et al*., 2018). In the case of this experiment, ewes № 9, 19, 20 are scored FAMACHA 4 and have high FEC; ewes № 5,10 are characterized by high FEC, but the FAMACHA score is 3; ewes № 6, 14 belong to the group with medium FEC and their FAMACHA score is changed from 4 in February to 3 in May. Again, the sensitivity of FAMACHA scoring is not reliable enough to predict the rise in FEC.

The correlation coefficient between FEC and FAMACHA was calculated and interestingly it was high enough: 0.416 in February and 0.813 in May. Therefore, statistically the two variables are positively correlated and should be considered together.

Estimating the effects of gastrointestinal parasitism based on reliable parameters such as FEC, FAMACHA scoring and hematology represents a promising approach to the prophylaxis of these economically relevant diseases. Decisions that help to lower the costs and reduce the losses in animals or production should be investigated and then incorporated into practice.

CONCLUSION

Results from the current study were able to identify the periparturient rise in ewes and perform a follow-up examination at the beginning of the grazing season based on the standard FEC technique. The introduction of the FAMACHA scoring system was aimed at as an additional tool in diagnostics. It can be recommended that these variables are estimated together with hematology to aid treatment decisions. Furthermore, routine testing can be utilized in the effort to select sheep that are possibly resistant or tolerant to gastrointestinal nematodes as a part of the sustainable parasite control approach.

CONFLICT OF INTERESTS

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