Ten years of gastrointestinal parasite monitoring in Zerasca Sheep

MARIA NOVELLA BENVENUTI[1]*, FABIO MACCHIONI[1], FRANCESCA PISSERI[2], EMILIA VENTURA[2], LORELLA GIULIOTTI[1]

[1] Department of Veterinary Science, viale delle Piagge, 2 26124 Pisa, Italy
[2] Veterinarian free lance

SUMMARY

The monitoring of gastrointestinal nematodes can aid in administering anthelmintic treatments limited to the effective cases. This approach can serve as a valuable tool for safeguarding animal welfare, the environment, and the quality of animals' derived products. Finally, yet importantly, it could be useful for containing the occurrence of drug resistance.

This study aimed to describe ten years of monitoring gastrointestinal nematode (GIN) burden in a farm of Zerasca sheep located in the North of Tuscany (Italy) which has not been treated with anthelmintics since 2012. Additionally, the study aimed to evaluate the influence of the date of sampling, the year, and the season on GIN and the correlation between the body condition score (BCS) and eggs per gram (EPG).

Faecal samples were collected to estimate the faecal egg count of GIN, expressed as eggs per gram (EPG), using a modified McMaster technique. BCS was checked using a scale ranging from one to five. Results of Fecal Egg Count were grouped into four levels of infection (0=0 EPG; 1=1-300 EPG; 2=301-600 EPG; 3=more than 600 EPG).

The study reveals significant fluctuations in gastrointestinal nematode burdens in sheep over ten years. The overall mean of EPG was 286.5 ± 620.28, with notable variations observed across sampling dates ($P \leq 0.001$). Although EPG levels varied over the years ($P=0.0002$), no significant differences were found across seasons ($P=0.9040$). The sheep population exhibited infestation predominantly falling within lower levels (0 and 1), but attention to health is warranted for the 14.7% exceeding 600 EPG. BCS and EPG showed a significant negative correlation ($r = -0.2295$; $p=0.0051$), indicating that BCS could serve as an indirect indicator of infestation levels. The study underscores the need to reevaluate GIN management strategies considering climate change and anthelmintic resistance. Targeted treatments for heavily infested animals, alongside maintaining anthelmintic susceptibility through refugia, are suggested as alternative approaches to widespread chemical treatments, which may contribute to resistance development and environmental contamination.

KEY WORDS

Sheep; Zerasca; gastrointestinal parasites; monitoring.

INTRODUCTION

Gastrointestinal nematode (GIN) infections can lead to significant productive losses resulting in weight loss, slow growth, reduced feed consumption, reduced milk production, and increased veterinary intervention costs, impacting the economy of farms (1, 2). Chemical anthelmintic drugs, such as benzimidazoles, imidazothiazoles, macrocyclic lactones, and amino-acetonitrile derivatives are the most used treatment for GIN infections (3). Unfortunately, an overreliance on these treatments without selection criteria, and the absence of a strategic control program have led to anthelmintic resistance (4, 5). To address and mitigate this issue effectively, farmers, in collaboration with technicians, should adopt alternative and complementary approaches thus avoiding the repeated use of pharmaceuticals. Parasite control should encompass alternative and integrated approaches, including limiting contact between hosts and infective larvae or eggs, through effective grazing systems management, ensuring proper nutrition, utilizing non-conventional therapies, stimulating of host responses, and modulating worm biology (6). An alternative approach to reducing anthelmintic treatment is the application of targeted selective treatments, which aim to treat only those animals showing signs of high infestation, such as low body condition score (BCS) and high FaMaCha (7).

Anyway, the initial step to establishing an effective control plan is regular GIN monitoring (8). This practice allows technicians to assess the risk to the health and welfare of the animals based on the effective presence of the parasite burden within the flock. It is important to emphasize that these alternative strategies contribute to meeting consumer demand for products with minimal chemical inputs and for animals raised with high welfare standards.

Sheep farming in Italy is extensively practiced, with some variations depending on the specific production address. A large number of animals and a high level of automation

Corresponding Author:
Maria Novella Benvenuti (novella.benvenuti@unipi.it)

characterize the sheep milk enterprise. Conversely, the meat enterprise is predominantly conducted on a smaller scale (9). In the Zeri valley (Massa Carrara, Italy), the breeding of the homonymous sheep represents an important sector of employment which is fundamental in limiting the progressive trend of human depopulation. Zerasca is a breed included in the list of endangered status (10) that counts little more than 2,000 heads (11). The meat of the heavy lamb is highly appreciated for its taste, which is attributed to the feeding sources based on pasture on grass and wood (12). In this area, farmers are motivated in the safeguarding of the breed, environment, and social tissue promoting balance between animal and environment. In this context, lamb meat reared in respect to environmental conditions could provide an additional source of income.

The small number of animals appears to facilitate a better control of the animals and in this context, human care could be a key to carry on a proper GIN control.

In any case, the control strategies must be based on the monitoring of the effective presence of parasite burden in the flock; this is fundamental to ensure a proper use of anthelmintics in the effective cases (13).

The aim of this study was to describe ten years of monitoring of GIN and BCS in a farm of Zerasca sheep that has not used anthelmintic treatments since 2012. Additionally, the study investigated the influence of seasons and sampling dates on GIN burden. Finally, the correlation between the body condition score (BCS) and Eggs per gram (EPG) was examined.

MATERIAL AND METHODS

The study was carried out on a farm of Zerasca sheep counting 50 heads located in the Zeri district (Massa Carrara, Italy) at an altitude of 800 m a.s.l. (44°19' N, 9°47' E). The Zerasca sheep is an indigenous Italian breed with endangered status. It is a sturdy animal of medium-large size with the attitude to meat production (Figure 1).

In Zeri, the warm season lasts about three months, from June to September, with a daily maximum temperature exceeding 19°C. The hottest month of the year is August, with an average maximum temperature of 21°C and a minimum of 15°C. The cold season spans four months, from November to March, with a daily maximum temperature averaging below 8°C. The coldest month in Zeri is January, with an average maximum temperature of 0°C and a minimum of 5°C. Rain falls throughout the year with a pick in October while the driest month is July (14).

Since over 20 years, the farmer collaborates with the Department of Veterinary Sciences of Pisa to limit chemicals use. This collaboration has involved implementing sustainable strategies such as rotational grazing, shelter hygiene, use of bioactive forages, and homeopathy. Animals are raised following an extensive management system where they are kept almost all year round on open pastures and grasslands. Sheep nourishment consists of grass, shrubs, bushes and other plants they come across during grazing on marginal lands and in wood. Animals have access to the pasture during good weather conditions and return to the shelter overnight for the presence of predators. Pasture mainly provides the ration but integration, consisting of corn and barley grain, is guaranteed daily. During particularly harsh climatic conditions, protein integration was supplied with pea or field bean.

Over a period of ten years (October 2012 - November 2022), the farm underwent inspection from two to four times a year to randomly collect faecal samples from the rectal ampule on 12 pluriparous ewes. The samples were stored in refreshing boxes and processed the following day. During the inspections, technician checked the body condition using the scale of Russel et al., (15) ranging from one (emaciated) to five (obese) while veterinarian checked the overall health status. Faecal samples were examined to estimate the faecal egg count of gastrointestinal nematodes, expressed as eggs per gram (EPG), using a modified McMaster technique (16).

Statistical analysis was performed by ANOVA with JMP software (17); one model included the date of sampling as variability factor, the other included the season. Data referring FECs were logarithmically transformed [$y = \log(EPG + 25)$] to normalize error (18). Results of FEC were grouped into four levels of infection (0=0 EPG; 1=1-300 EPG; 2=301-600 EPG; 3=more than 600 EPG) in order to point out the potential risks of the parasite burden (19, 20). The relation between BCS and EPG level was performed by Spearman's RHO test.

RESULTS

The overall mean of EPG was 286.5 ± 620.28. Statistically significant fluctuation of EPG in relation to the sampling date was recorded ($P \leq 0.001$). An unusual peak of EPG (504)

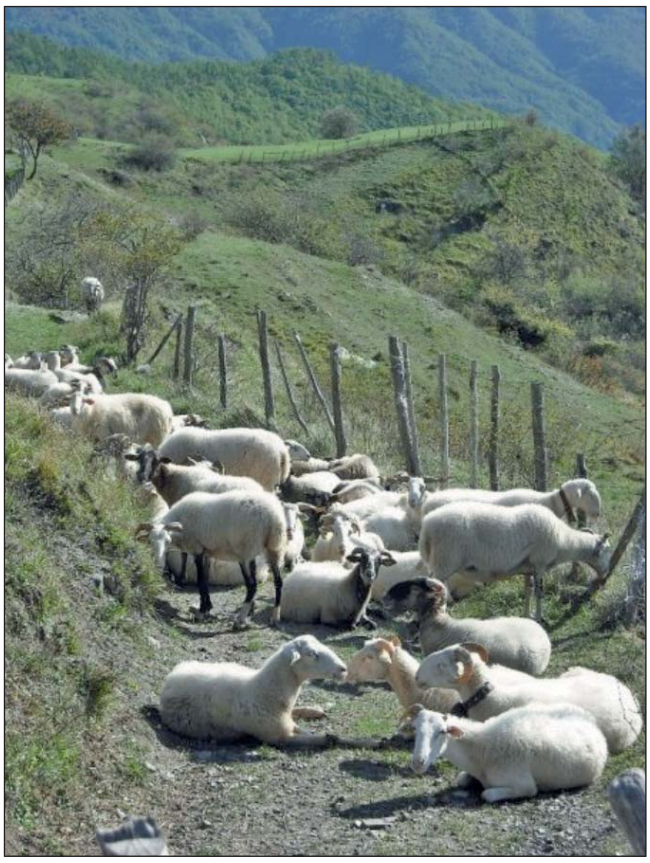


Figure 1 - A flock of Zerasca sheep

Table 1 - EPG mean referred to each sampling.

		EPG		BCS	
Sampling	Date	Mean	SE	Mean	SE
1	October 2012	614.29	120.977	3.2	0.135
2	December 2012	100.00	160.037	3.2	0.313
3	February 2013	88.89	150.885	3.0	0.236
4	April 2013	341.82	136.480	3.1	0.214
5	June 2013	88.57	120.977	3.3	0.221
6	August 2013	504.00	143.142	3.1	0.150
7	October 2013	189.09	136.480	3.1	0.097
8	December 2013	146.67	150.885	3.0	0.000
9	February 2014	164.44	150.885	3.2	0.206
10	April 2014	400.00	171.087	2.9	0.092
11	July 2014	146.67	150.885	3.0	0.000
12	October 2014	152.00	143.142	3.0	0.000
13	March 2015	526.67	130.670	2.9	0.065
14	June 2015	40.00	136.480	3.0	0.000
15	October 2015	248.00	143.142	2.8	0.076
16	January 2016	188.00	143.142	3.0	0.000
17	June 2016	325.71	120.977	2.9	0.057
18	October 2016	218.18	136.480	2.9	0.061
19	January 2017	451.43	120.977	2.8	0.066
20	April 2017	160.00	150.885	3.0	0.000
21	September 2017	730.00	130.670	2.8	0.074
22	January 2018	228.33	130.670	3.0	0.042
23	April 2018	177.78	150.885	3.0	0.000
24	August 2018	340.00	143.142	3.0	0.000
25	October 2018	141.54	125.544	3.0	0.000
26	December 2018	204.00	143.142	3.0	0.000
27	March 2019	705.45	136.480	2.9	0.070
28	July 2019	378.18	136.480	2.8	0.170
29	November 2019	298.18	136.480	2.9	0.184
30	February 2020	360.00	130.670	2.9	0.120
31	October 2020	301.82	136.480	2.9	0.045
32	February 2021	334.55	136.480	3.1	0.132
33	May 2021	665.00	160.037	3.0	0.134
34	March 2022	440.00	160.037	2.9	0.157
35	November 2022	294.55	136.480	2.9	0.157

was observed in August 2013 (Table 1). Additionally, statistical differences of EPG during the years were detected (P=0.0002) (Graphic 1). No significant differences (P=0.9040) in the variation of GIN burden were observed across seasons (Table 2).

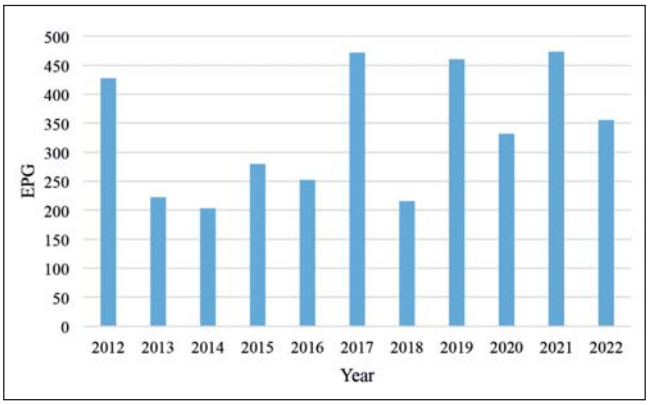
The intensity of the GIN burden in the flock throughout the entire study period is illustrated in Graphic 2. Sixty-eight percent of the sheep exhibited infestation levels falling within levels 0 and 1, while only 14% of the sampled population exceeded the threshold of 600 EPG, indicating the need for attention to health and suggesting that the administration of chemical treatment may be warranted (21).

The overall mean of BCS resulted 3.0 ± 0.40.

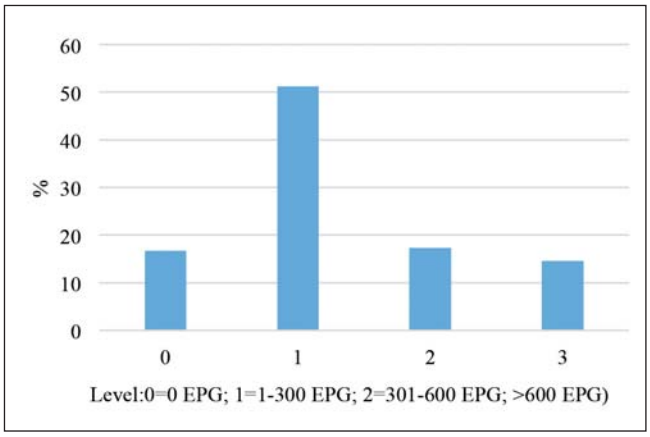
BCS and EPG were significantly negatively correlated (r = -

Table 2 - EPG fluctuation in relation to the seasons.

	EPG	
Season	Mean	SE
Winter	244.04	46.155
Spring	359.07	45.292
Summer	342.22	64.053
Fall	329.12	44.084



Graph 1 - EPG mean referred to each years.



Graph 2 - GIN burden levels distribution.

0.2295; p=0.0051). Arising the level of EPG, BCS decreased pointing out that BCS can be considered an indirect indicator of the level of infestation (7).

DISCUSSION

In examining the gastrointestinal nematode (GIN) burden within the Zerasca sheep population, our study embarked on a decade-long exploration, a duration not commonly observed in similar research endeavors. The overall mean of EPG was comparable to that reported in other studies on Zerasca sheep (22; 23), except for the standard deviation, which exhibited higher variability. This increased variability can be attributed to the duration of the study. A longer observation period could allow capturing a broader range of environmental and seasonal conditions, thus contributing to increased variability in the data.

The ten-year duration of the investigation on chemically untreated sheep is not commonly found in other studies, making comparisons difficult.

It is noteworthy that the eggs output did not consistently follow the typical trend (19). Despite high levels of EPG being noted during periods associated with the well-known spring and fall rise phenomenon (sampling 1, 21, 27, 33), considering the entire long study period, no significant differences (P=0.9040) in the variation of GIN burden in relation to the seasons were observed. Furthermore, an unusual peak of EPG (504) was observed in August 2013. In support of this occurrence, the buildup of infectious L3 larvae speeds up at higher temperatures due to shorter reproduction cycles, resulting in a higher number of parasites and increased disease risk from midsummer onwards (24). The current management of gastrointestinal nematodes (GIN) in sheep relies on epidemiological studies that are becoming outdated due to climate change which can lead to shifts in the prevalence and distribution of GIN, affecting traditional epidemiological models, and the widespread emergence of anthelminthic resistance that poses a significant challenge to traditional management strategies. Consequently, the prevailing beliefs about the epidemiology of GIN at the farm level need to be re-evaluated to challenge the understanding of sustainable control principles and their practical application (25).

Anyway, some studies such as Salehi et al. (26) reported significant variation in parasitic burdens across different seasons. The Authors found that fall had the highest rate of infection, followed by summer and spring, with lower infection rates observed in winter.

In our study, 85.3% of the sheep showed an infestation level under 600 EPG, with only 14.7% of the samples exceeding the threshold considered dangerous to health. In this situation, the administration of chemical treatments to the whole flock could represent a threat of anthelmintic resistance and the worthless dissemination of environmental contaminants. In such cases, it may be useful to consider the possibility of accomplishing target treatments as suggested by some Authors (27). This approach aims to treat only those animals showing signs of high infestation, such as low BCS and high FaMaCha (7). In particular, BCS≤2 is considered the best criterion to detect ewes with FEC≥750 EPG (28, 29). A farm typology that has a limited number of heads could easily handle this procedure.

Furthermore, the literature emphasizes the importance of leaving a proportion of the worm population unexposed to treatment to maintain anthelmintic susceptibility (refugia) and limit the development of anthelminthic resistance (30). It is also crucial to consider that an animal without worms may not be beneficial, as the presence of worms can stimulate the development of animal resistance to parasites (31).

CONCLUSIONS

In conclusion, the ten-year study on a Zerasca sheep farm that refrained from using anthelmintic treatments yielded noteworthy and significant findings. The research revealed significant fluctuations in gastrointestinal nematode egg output, with certain peaks occurring unexpectedly, challenging conventional seasonal patterns. Despite these fluctuations, the long-term observations suggested a generally balanced distribution of GIN burdens across seasons but variations in GIN burdens over the years were detected. The negative correlation observed between BCS and EPG could serve as an indirect but useful indicator of GIN infestation levels.

The implementation of a successful parasite monitoring program not only benefits both the animals and the environment but also provides a cost-effective advantage. The prolonged ten-year duration of this study, a rarity in the field, not only sheds light on the challenges of comparisons with shorter investigations but also highlights the need for long-

term studies in an era of climate change and anthelmintic resistance.

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Declaration of Competing Interest
The authors declare no conflict of interest.

Ethical approval
All animal procedures used in this study were in compliance with the ethical and animal welfare concerns of the Committee on the Ethics of Animal Experiments of Minimally Invasive Surgery Centre and fully complied with recommendations outlined by the Italian laws.

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