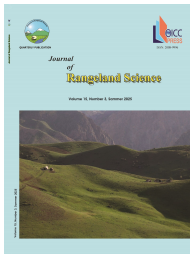

Volume 15, Issue 3, 152520 (1-8)

Journal of Rangeland Science (JRS)

https://doi.org/10.57647/j.jrs.2025.1503.20

Effect of sheep grazing management on the endoparasite population on grassland

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Original Research

Received:
22 November 2023
Revised:
14 March 2024
Accepted:
19 August 2024
Published online:
20 July 2025

Abstract:

Our research objective was to clarify the effect of different sheep grazing practices on parasite infestation in a semi-natural grassland community with a solonyec soil composition. Sampling for the occurrence of internal parasites of sheep was carried out in three extensively cultivated *Achilleo-Festucetum pseudovinae* grass communities with different grazing systems but in the same site conditions, in 2022 – 2023, at the MATE Research Institute in Karcag, Hungary. The sheep grazing regimes were: pastoral grazing, rotational grazing, and permanent, shelterbelt-forest grazing used in all grazing days. Microscopic analyses of the genomes and numbers of potentially infective oviposition and L3 larval stage endoparasites in sheep pastures were carried out on samples of faecal matter from grazed pastures and grass samples prepared using a 'larval running'. During our investigations, we found that the most infested area was the permanent pasture, 1350 eggs/g were found and *Trichostrongylida* type eggs were the most common in the faecal samples; furthermore, *Nematodirus* sp., *Eimeria* sp. and Strongylid type eggs were the most highly ones in this area.

Keywords: Sheep; Endoparasitic; Grazing mode; FLOTAC technique; Larval running

Introduction

At the international level, the presence of internal parasites in flocks is a permanent or seasonal problem in almost all regions where sheep are kept. As very serious economic losses can occur, sheep farmers have to regularly calculate significant costs for the treatment of sick sheep and for protocol-based preventive medication.

The problem is exacerbated by the recent increase in the construction of pasture gardens on pastures still in use due to the chronic shortage of animal carers. This method is labor-saving but if it is not used professionally, it can lead quite quickly to the degradation of grassland vegetation and it will be a breeding ground for endoparasites (Asif et al., 2008; Taylor, 2010), which adhere to the vegetation and await host animals in an infective L3 larval stage, according to multi-year studies by Monori et al. (2019) emphasizing that sheep are infected with larvae while grazing with negligible infection in barns. Therefore, pasture vegetation may be a potential source of infection, one of the reasons for the change in the traditional sheep husbandry system, and more specifically, the cessation of daily grazing rotation, in parallel with the devaluation of the pastoral culture based on ancestral experiences (Tóth et al., 2018a, 2018b).

According to Coffey et al. (2007), Maxwell (2008), and Coffey and Hale (2012), a critical point in the parasite issue is the length of time it takes to release animals onto the same pasture. Sunlight and summer drought can be beneficial in breaking the infection chain.

Monori et al. (2019) conducted endoparasite monitoring using faecal samples from Hungarian Merino genotype ewes kept on the pasture without any anti-parasitic treatments for the 5th year, and found that the level of infection increased from 5 – 6 weeks after April turn-out, peaking in early summer and then slowly declining until October when it started to increase. At the end of the sheep grazing season, the infestation slowly decreased in the flock trough, stabilizing at a mild level during the winter. Based on their studies, it is recommended that interventions against internal parasites should not be carried out routinely, but should be based on the pest risk depending on the season. Based on Monori et al. (2019) over 5 years in arid, extensive grassland with

arid, solonyec soils, the following endoparasitic genera are highlighted in order of frequency:

- Gastrointestinal parasites (Trychostrongilodosis): high infestation in early summer and late autumn.
- Fungal lungworm disease: persistent parasite, even after treatment.
- Tapeworm (*Moneiza* sp.): in growing stock, regularly caused severe loss of condition in spring.
- Strongyloidiasis: a persistent infection with mild symptoms:
- Dicroceliosis: only in spring a slight infection was observed.
- Coccidiosis: a persistent infection was detected, but no symptoms were observed in the adult sheep flock.

Varga et al. (2020) and Varga et al. (2021) recommend the use of a larval feeding device to assess the endoparasite infestation of extensive grassland on saline soils to assess the level of infestation in the grassland, where the number of larvae can be detected from the cut phytomass.
Varga et al. (2022) investigated the use of tannin extracts from the roots of *Cichorium intybus var. foliosum* as a conditional herding practice in confirmed infested ewes. It was found that a clear reduction in Strongylus species was measurable as a result of the extract.

The development of parasites

The adult worms deposit the eggs in the faeces of the animal's intestinal tract, from which after defecation in the pasture, the L1 and then the L2 larvae develop. The next stage is the development of L3 infective larvae, which take on average 4 days to develop, depending on the genome. The cuticle of the L2 larva remains as a protective sheath. During this period, they do not feed but use stored food to survive. The third type (L3) larvae migrate to their immediate environment in warm and humid weather, where they are eaten by grazing animals along with the grassland vegetation (Blackburn et al., 2011). Then, they develop into adults in about 14 days, completing their life cycle. The time between ingestion of the larvae and the emergence of the eggs is about 16 to 21 days (Peregrine et al., 2012).
Development between eggs and L3 larvae can take up to 12 weeks in early spring and up to 7 days in summer (Varga et al., 2020). The life cycle differs in some species. In *Nematodirus* species, the L3 larvae develop in the oviposition, which takes $2-3$ weeks (Kassai, 2003).
Connor et al. (2007) investigated the larval stages of free-living larvae between species and within species. The minimum time for eggs to develop into L3 larvae is $3-4$ days when the following environmental conditions are optimal: temperature and humidity (Aboagla and Maeda, 2011). Cold temperatures have an adverse effect on *Trichostrongylus colubriformis* species. In addition to temperature, the effect of humidity on the development of the worms has also been investigated. Connor et al. (2006) studied interspecific as well as intraspecific free-living larval stages. The minimum time for eggs to develop into L3 larvae is 3 to 4 days when the following environmental conditions are optimal: temperature and humidity. Cold temperatures have an adverse effect on *Teladorsagia circumcincta* and *T. colubriformis* species. Furthermore, it was found that the temperature and humidity of the faeces greatly influence the development of L3 larvae from the egg. *T. circumcincta* is the most resistant to moisture.
Connor et al. (2006) found that *T. colubriformis* migrates more rapidly from the faeces to the lawn, while individuals of *T. circumcincta* can remain in the faeces for up to 10 months. Barger et al. (1984) found that *T. colubriformis* larvae can remain in the faecal matter for up to 18 months. The highest concentration of larvae was found at the end of the drought. It was also found that cool, dry weather prolongs the life of the larvae and hot, humid weather shortens their life. Connor et al. (2006) found the life span of *T. colubriformis* was 8 days.
Miller et al. (1998) found that the parasites must develop before the infective stage in order to emerge from the guts into the pasture where they can be consumed by sheep along with the grass. The development of *T. colubriformis* larvae occurs between 10 and 36 °C. *T. colubriformis* species prefer cooler and drier conditions. They infest during two periods, March-June and September-November.
Our research objective was to clarify the effect of different sheep grazing practices (pasture grazing, intermittent grazing and pasture garden grazing) on parasite infestation in a semi-natural grassland community with a Solonyec soil condition.

Materials and methods

Description of the location

Our studies were carried out in the autumn of 2022 and spring of 2023 on the grassland plot 01712/1 of the MATE Research Institute in Karcag (figure 1). The grassland association is *Achilleo-Festucetum pseudovinae*, the soil type is medium meadow-colony, the elevation is 91 m a.s.l. The mean temperature and monthly precipitation during the study period are presented in Table 1.
The ewes used in the experiment were of the Hungarian Merino and Blanc du Massif Central F1 genotypes, aged between 2 and 6 years, with a flock size of 380. We did not have the opportunity to pull in exotic sheep breeds. Each grazing system was tested on 10 ha of grassland, also with 380 ewes. No wild animals (rabbits, foxes, and deer) capable of spreading the infection enter the areas studied. We had the opportunity to compare three different grazing regimes (treatments) under the same site and floristic conditions:

1. Pastoral grazing: sheep flocks return every 12 days for one day to the grassland under study.
2. Rotational, enclosed pasture grazing: sheep return to the pasture every 12 days for 5 days.
3. Permanent pasture, with daily use, as this is where the flock's dolphin forest is located.

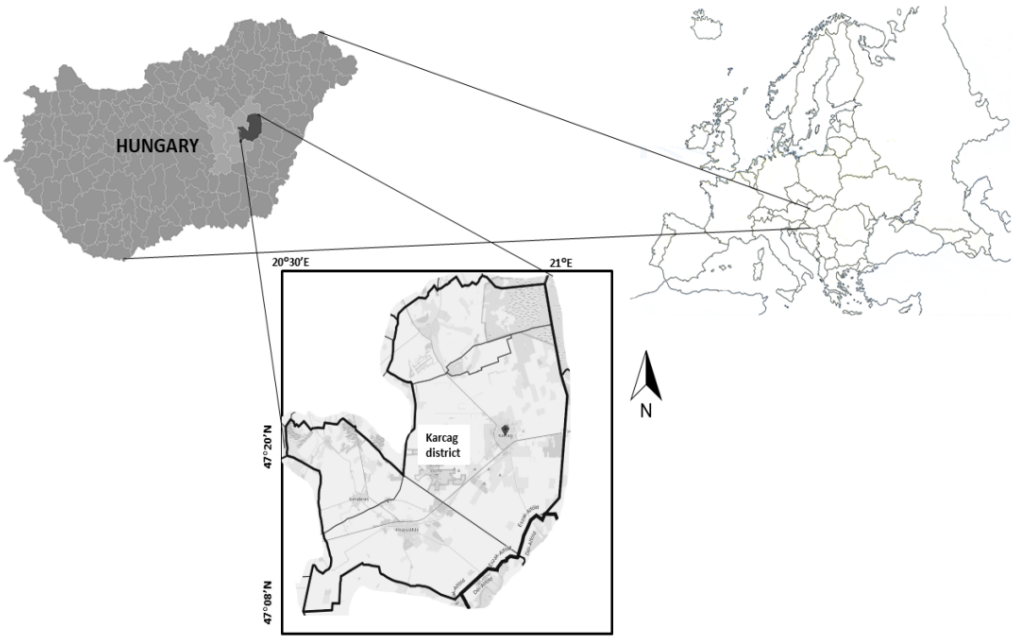

Figure 1. The location of the study area, Hungary, Karcag.

The Agro-Ecological Program will be in force in Hungary from 2023 (Decree 15/2023 (19.IV.) AM), which specifies (§ 21 – 22) that grazing in one section may not exceed 12 days. It can be seen that in the case of grazing 1 and 2 (management), we followed the legislation and respected the minimum times for grazing and grazing regeneration. For the treatment 3 as the permanent pasture, we followed the grazing regime that is widely used by farmers not participating in environmental programs. It should be noted that the main compelling reason for this unskilled, grazing use is the critical shortage of pastoral labor in the European Union.

In the autumn of 2022 (14 Nov. 2022), faecal samples (10 samples) were collected from the study sites and examined microscopically. Fresh faecal samples, up to 1 day old, were collected from each site, which eliminated the possibility of having different stages of development of parasitic eggs. Faecal samples were taken randomly in the pasture. Microscopic examination was carried out on the same day as the faecal collection.

Mini-FLOTAC (chamber) and Fill-FLOTAC-5 were used to analyse faecal samples (Cringoli et al. (2017); figure 2). This test method is rapid, taking only about 12 – 15 min to prepare the sample. The test can also detect parasitic eggs and larvae. First, the faecal sample was homogenized using a spatula, then 5 g of faecal sample was mixed with 45 mL of 50% NaCl saline solution in the Fill-FLOTAC (homogenization). From this homogenised solution, 1 – 1 mL of homogenised solution was filled into both chambers of the Mini FLOTAC using a pipette. After floating the eggs (10 min waiting time), the *Trichostrongylida*-type and other eggs were counted under a microscope in the chambers. The

Table 1. Climatic data for the experimental period (Karcag 2022-2023).

Year	Month	Mean temperature (°C)	Precipitation (mm)
2022	September	16.11	64.9
	October	12.54	2.8
	November	6.48	36.9
	December	2.46	81.1
2023	January	4.3	60.1
	February	2.6	6.8
	March	7.4	34.5
	April	9.5	39.7
	May	16.54	49.9

Source: MATE, Karcag Research Institute.

Figure 2. Fill-Flotac-5 (by Krisztina Varga).

sensitivity threshold of the assay method is high (5 eggs/g), so the number of counted eggs multiplied by 5 gave the EPG value (egg/g) of the number of parasitic eggs in 1 g of faecal sample. Morphological determination of eggs was based on Kassai (2003).

In the spring of 2023, grass samples were collected on two occasions (April 5 and June 1st, 2023), followed by a larval run (figure 3) and microscopic examination to determine the infestation of the areas. The grass samples were collected randomly from the pasture gardens. From each pasture garden, 2 kg of clippings were collected. The larval funnel we used was modeled on the Tullgreen apparatus (Twardowski et al., 2004). The funnel measures 67 cm in diameter, 93.84 cm in height, and 117.4 liters in capacity. The funnel is made of transparent, flexible axpet plastic and placed on a stand. The bottom of the funnel (5 cm in diameter) was covered with a mosquito net to prevent grass clippings from falling into the container and a 5-liter empty container was placed underneath. The grass sample placed in the funnel

Figure 3. Larval running devices with infra lamps (Made by: Varga Krisztina).

was washed with 2 liters of distilled water and the leachate was collected in the container. An infrared light was placed over the already-washed grass sample in the funnel so that the larvae moved lower and lower in the drying sample in search of moisture until they finally fell into the container. At the end of the larvae run, when the cut phytomass had dried, we washed the phytomass in the funnel with 2 liters of distilled water once more to ensure that any larvae that died during the larvae run were also included in our dish. The liquid in the dish was left to settle for 12 hours and the number of larvae was determined by microscopy. The time of the first larvae run was between May 4 to 10 in 2023, the larvae counting under the microscope was done on May 11, 2023. The time of the second larvae run was between 1st and 7th June 2023, the larvae counting under the microscope took place on 8th June 2023. Faeces and grass samples were taken in the morning. For the microscopic examination, 10 $\times$ 1 mL of liquid was used, which was extracted from the bottom of the dish using a pipette. The larvae were not identified by morphological characteristics as unfortunately, no veterinary reference is available in Hungary. At the same time, no faecal samples were taken, but this will be done in the continuation of the experiment.

For microscopic examination, we used a Delta® Optical microscope with 10x magnification (10/0.25 x 160/0.17) to count endoparasitic larvae and eggs.

Statistical analysis

The data were collected then were processed using Microsoft® Office Excel.

One-way analysis of variance (ANOVA) was used for statistical analysis of the data. Analysis of variance was used to determine whether there was a significant difference between the means of the three groups. It is important to note, however, that this statistical analysis does not show where the difference between the means of the groups lies.

Analysis of variance was conducted to compare the three different types of pasture for endoparasitic infection based on the number of eggs found in the faecal sample. The Least Significant Difference (LSD) test, also at a 5% significance level, was used to test the differences between treatments.

Results

Egg count test results

Sheep faeces samples were taken from three different grazed grassland areas. The collected samples were used for ova count analysis after sheep grazing was completed in November 2022. This is a preliminary test for parasites on the grassland. During our examination, we found parasitic species of *Eimeria* sp., *Nematodirus* sp. and eggs of *Trichostrongylid* type and Strongylid type. Neither *Heamochus concortus* nor larvae were found in any of the areas during the examination of faecal samples. We found that most of the eggs were found in the permanent pasture during microscopic examination. The area with the lowest number of eggs was found in the pastoral grazing area, with a total of 49 eggs in the 10 samples. In the intermittent grazing area, 108 parasite eggs were counted in the 10 samples. A total of 328 endoparasitic eggs were found in the 10 samples from

the permanent grazing area, making this the most infested area. For each parasite species and egg type, the permanent pasture showed the highest infestation, i.e. the highest number of eggs counted. The intensity of infestation of the different species in the grasslands exploited with the three different grazing systems was compared and is shown in figure 4.

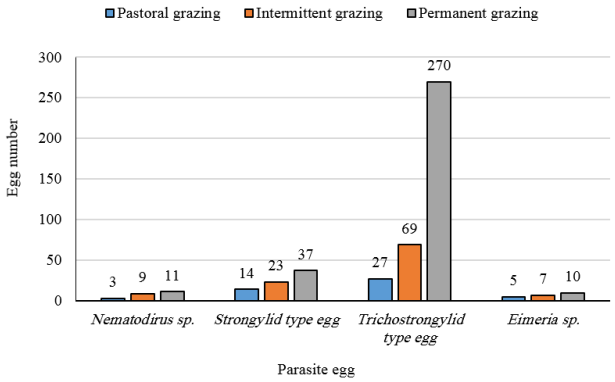

Figure 4. Intensity of parasite infestation in different areas.

We calculated the number of eggs/g found in samples from each pasture (EPG, Table 2). In the Table, the EPG number of samples is indicated in bold. The sensitivity of the FLOTAC method is 5, so the total number of eggs found after microscopic examination was multiplied by 5 to obtain the number of eggs per gram. During our tests, we found that the highest infestation of *Trichostrongylid* type eggs was found in all three pastures. In the pasture grazing area 135 eggs/gram, in the intermittent grazing area 345 eggs/-gram and in the permanent grazing area 1350 eggs/gram of *Trichostrongylid* parasitic eggs were found in one gram of faecal sample. In the pastoral grazing area, 70 eggs/g, in the intermittent grazing area 115 eggs/g and in the permanent grazing area 185 eggs/g Strongylid parasitic eggs were found in one gram of faeces. In the pastoral grazing area: 25 eggs/g, in the intermittent grazing area: 35 eggs/g and in the permanent grazing area: 50 eggs/g of *Eimeria* sp. parasitic eggs were found in one gram of faeces. In the pasture, grazed area 15 eggs/g, in the intermittent grazed area 45 eggs/g and the permanent grazed area 55 eggs/g of *Nematodirus* sp. parasitic eggs were found in one gram of faeces. The parasite infestation according to Kassai (2003) indicates that the herd was low infested.

Analysis of variance showed significant differences between three different types of pasture for endoparasitic infection based on the number of eggs found in the faecal sample

Table 2. Number of eggs and eggs/g in the study areas.

Grazing regime	Sample no	*Nematodirus* sp.	*Strongylida* type egg	*Trichostrongylid* type egg	*Eimeria* sp.	Sum of eggs	EPG (egg/g) Sample*
Pasture grazing	1	0	1	3	1	5	**25**
	2	0	1	1	0	2	**10**
	3	1	2	4	1	8	**40**
	4	0	1	3	0	4	**20**
	5	1	2	3	0	6	**30**
	6	0	0	2	0	2	**10**
	7	0	2	4	1	7	**35**
	8	0	3	3	1	7	**35**
	9	1	1	3	1	6	**30**
	10	0	1	1	0	2	**10**
Intermittent grazing	1	2	2	6	1	11	**55**
	2	1	3	8	1	13	**65**
	3	0	1	5	0	6	**30**
	4	0	2	7	1	10	**50**
	5	1	3	7	1	12	**60**
	6	1	2	9	1	13	**65**
	7	1	2	6	0	9	**45**
	8	2	3	8	1	14	**70**
	9	1	2	8	1	12	**60**
	10	0	3	5	0	8	**40**
Permanent grazing	1	0	3	28	0	31	**155**
	2	2	3	31	1	37	**185**
	3	0	4	25	2	31	**155**
	4	2	5	27	1	35	**175**
	5	2	4	38	1	45	**225**
	6	1	2	29	1	33	**165**
	7	2	5	32	2	41	**205**
	8	0	3	3	2	8	**40**
	9	1	5	28	0	34	**170**
	10	1	3	29	0	33	**165**

* = the number of counted eggs multiplied by 5.

($p < 0.01$) (Table 3). So, we performed the LSD test ($p < 0.05$). We found a significant difference between herding and permanent grazing and between intermittent and permanent grazing. Furthermore, we examined the groups of parasites found between different sheep grazing regimes.

Results of the larval survey

During the larvae run, we examined the infestation of L3 infective larvae in spring and late spring (April 12 and May 4, 2023) at two different times in the three different grazing areas. The results are shown in Table 4. During the study, we found that the spring was rainy. Infestation increased in all three areas during the study period (on May 4th). The highest infection was found in the permanent grazing area. The number of endoparasitic infective larvae increased on April 5 and June 1 were 4 and 10 (for pastoral grazing), 6 and 11 (for intermittent grazing), and 14 and 21 (for permanent grazing), respectively (Table 4). In the spring survey, 4 larvae were detected in 1 mL samples in the pastoral grazing area, and 10 larvae in the late spring survey. Overall, the lowest number of larvae was measured in the pasture grassland under the grazing grazing method. In the intermittent grazed area, 6 and 11 larvae were detected. In the permanent grazed area, 14 larvae were found in April and 21 in May.

Discussion

In our manuscript, we confirmed the findings of Asif et al. (2008) and Taylor (2010) that permanent pastures with daily use are a potential source of endoparasites that can be transmitted from grassland vegetation. Based on the FLOTAC technique, the endoparasite content of faecal samples from the permanent pasture was 1350 eggs/g *Trichostrongylid* type of egg, 185 egg/g *Strongylida* type of egg and 50 egg/g *Eimeria* sp. genomes, while in pastoral grazing, these genomes were reported as 135-70-25, respectively. The numbers indicate a risk factor for the rapid expansion of permanent, fixed grazing systems in grazing-based ruminant livestock production due to cost and labor efficiency.

A critical point in the endoparasitic status of pastures is the time taken for livestock to return to a given grazing section. In our rotational, fixed pasture management, we found six larvae in early April and 11 in early June, while in the pasture section with daily grazing, we found 14 larvae in early April and 21 in early June, almost half the number of larvae that threatened sheep, due to the 12-day grazing rest period, similar to the results of Maxwell (2008) and Coffey and Hale (2012).

Remarkably, and warranting further research, we found that the endoparasitic rainfall, the lowest number of larvae increased the highest (150%), similar to the results of Van Dijk et al. (2009) who emphasize that monitoring parasite infestation in pastures is of paramount importance for animal welfare and the quality of animal products for human consumption.

Comparing our results with those of Monori et al. (2019) under similar site conditions and similar extensive sheep grazing techniques in the same landscape, we found that *Trichostrongylus* and *Strongylus* genera were consistently

Table 3. Number of eggs and eggs/g in the study areas.

Grazing regime	*Nematodirus* sp.	*Strongylida* type egg	*Trichostrongylid* type egg	*Eimeria* sp.	Sum of eggs	EPG (egg/g) Sample
Pasture grazing	3b	14c	27b	5a	49c	245c
Intermittent grazing	9a	23b	69b	7a	108b	540b
Permanent grazing	11a	37a	270a	10a	328a	1640a

In each column, means followed by the same letter are not significantly different according to LSD ($P < 0.05$)

Table 4. Number of larvae found in the three grazing areas on two dates April 5 and June 1, 2023.

Sample no	Pastoral grazing		Intermittent grazing		Permanent grazing	
	April 5	June 1	April 5	June 1	April 5	June 1
1	0	1	0	1	2	3
2	1	2	1	2	1	1
3	0	0	0	1	2	3
4	0	1	0	1	2	3
5	1	2	2	2	1	1
6	1	1	1	2	2	4
7	0	1	0	0	2	3
8	1	1	1	1	1	2
9	0	0	0	0	0	0
10	0	1	1	1	1	1
Total	4	10	6	11	14	21

present in pastures in both sheep endoparasitic studies. However, while in our experiment, the genus *Eimeria* was present alongside the former genera during spring recruitment, studies by Monori et al. (2019) indicated a severe loss of condition due to *Moneiza* sp.

Conclusion

Monitoring endoparasitic populations on the grazed grassland that pose a threat to livestock and keeping them under threat is essential for all livestock keepers. Two further trends justify the high practical relevance of this research topic. Firstly, the increasing international trade in breeding animals may introduce endoparasitic species into our breeding facilities that have not been seen before, leaving our animals unprepared for their infection. A key issue is the timely detection of these parasitic species before their possible graduation. On the other hand, due to the lack of precipitation caused by climate change, intensive ruminant species should be provided with irrigated grassland in grazing-based management systems, and our manuscript confirms the fact that with better water supply, there are higher numbers of endoparasites in the field, so more care should be taken to monitor the endoparasite status of the area.

As every year is different, one of the prerequisites for effective grazing livestock management is to find the optimal time for preventive veterinary control of endoparasites and to intervene when there is a risk of damage to reduce the development of resistance to the agent.

Authors Contributions
All authors have contributed equally to prepare the paper.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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