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Prevalence and Risk Factors of Gastrointestinal Parasitic Infections in Small Ruminants in the Greek Temperate Mediterranean Environment

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ABSTRACT

Gastrointestinal (GI) parasitic infections of sheep and goats were investigated in 69 farms located in Thessaly region of Greece, characterized by temperate Mediterranean climate, during two consecutive seasons. A total of 557 fecal samples were collected. Helminth eggs were detected in 44 (7.9%) samples. Strongyle-type eggs were found in 19 (3.4%) samples, *Nematodirus* spp. eggs in 6 (1.1%) samples, *Trichuris* spp. eggs in 16 (2.9%) samples, *Fasciola hepatica* in 3 (0.5%) samples, and *Dicrocoelium dendriticum* in 1 (0.2%) sample. Coccidian oocysts were found in 36 (6.5%) samples. Risk factors related to animal and farmer status, farm and pasture management, and environmental factors derived by satellite data were examined for their association with the prevalence of helmith infections. A logistic regression model showed that the educational level of farmers and the elevation of farm location were associated with helminth infections.

Keywords: Risk Factors; Gastrointestinal Parasites; Sheep; Goats; Farm; Greece

1. Introduction

Small ruminant farming is an important economic activity for European countries, especially those with a temperate Mediterranean environment and particularly for Greece. Sheep and goats are infected with the same principal gastrointestinal nematode species, which provoke similar pathological changes and economic consequences [1]. The parasitism with helminths, and particularly nematodes of the gastrointestinal tract, is a major threat for ruminant production, health and welfare associated with outdoor breeding [2]. Specifically, gastrointestinal (GI) parasites are one of the main constraints to small ruminant production in temperate countries [3] and may amount to 20% loss for marketable products [4]. The traditional sheep production system in Greece is extensive without transhumance where sheep stay in permanent installations near the villages and graze nearby [5]. Control of helminths in Greece is currently based exclusively on the frequent use of anthelmintics. However, the appearance of anthelmintic resistance in Greece [6,7] indicates the need for control strategies targeting only animals at high risk for GI infections in order to limit the use of anthelmintic drugs and conserve their efficacy.

Previous studies have investigated the epidemiology and importance of GI parasitic infections in small ruminants, but few studies have addressed the role of animal, farm, and environmental factors in relation to the presence of GI parasitic infections in small ruminants in the Mediterranean area [8-11]. The objective of the present study was to examine the animal, farm, and environment related factors that may affect the risk for GI parasitic infections of small ruminants in Greece, where no previous studies have been reported.

2. Materials and Methods

2.1. Animals and Sampled Areas

Fecal samples were collected from clinically healthy and randomly selected sheep and goats in organic and neighboring conventional farms. These farms are registered with the Hellenic Ministry of Rural Development

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and Food according to the latest available census (2005) in the region of Thessaly, Greece. The region of Thessaly is located in Central Greece (**Figure 1**). It is one of the largest sheep and goat producing areas of Greece and accounts for 12.5% of the total sheep and goat production in Greece (data for 2006 provided by the National Statistical Service of Greece). In addition, 28% of organic sheep and goat farming in Greece is located in Thessaly (data for 2005 provided by the Hellenic Ministry of Rural Development and Food). Thessaly is generally affected by a temperate Mediterranean climate which is characterized by dry summers with occasional precipitation and calm, wet winters. There are droughts during the summer months.

Farms whose owners agreed to participate in the study were visited once between September 2006 and February 2007 and were equally distributed by autumn and winter seasons. Fecal samples (10 - 20 g) were collected directly from the rectum of the animals and stored at 4˚C until analyzed. Data on animal characteristics, farm management practices, and farmer status were collected through a survey questionnaire at the time of sampling. Data were collected via a 2-page questionnaire comprising 10 closed questions. In order to avoid any misunderstanding, the investigators completed the questionnaires by interviewing the farmers at the time of the visit to the farm for sample collection. The questionnaire with pre-coded replies is available on request by e-mail.

2.2. Parasitological Analyses

Fecal egg counts (epg) were conducted using the McMaster method [12] separating strongyle-type eggs, *Nematodirus* spp., *Trichuris* spp., *Fasciola hepatica*, *Dicrocoelium dendriticum* eggs, and coccidian oocyts. The flotation medium used for nematode and cestode eggs was sugar solution with a specific gravity of 1.2, while for trematode eggs was zinc sulphate with a specific gravity of 1.5. To increase the accuracy of the McMaster method four chambers per sample were enumerated.

2.3. Source of Environmental Data

Environmental data for farm locations were obtained from the MODIS (Moderate Resolution Imaging Spectroradiometer) instrument aboard the Terra (EOS AM) satellite (https://lpdaac.usgs.gov/), products MOD13C2 and MOD11C3, with a resolution of 0.05 deg for the land surface temperature (LST) and the normalized difference vegetation index (NDVI). Rainfall data was extracted from the 3B43 rainfall product of the Tropical Rainfall Measuring Mission (TRMM) satellite (http://disc2.nascom.nasa.gov/) with a resolution of 0.25 deg. NDVI and LST were hypothesized to represent surrogate measures of environmental moisture and temperature, respectively [13]. The environmental data were recorded as monthly means for 12 months before the day of sampling for each examined farm. Geographical coordinates and elevation data of farm locations were obtained from the Digital Elevation Model (DEM), SRTM30 dataset for Greece with a resolution of 1km and CGIAR-SRTM data (http://srtm.csi.cgiar.org/) aggregated to 30 seconds. The inland water digital chart of the world (http://www.diva-gis.org/gdata) was used for the construction of the geographic features of Thessaly.

2.4. Statistical Analysis

The relationship between the prevalence of helmith infections and the investigated factors was analyzed by defining a binary outcome variable where positives samples (as determined by coprological examination) were coded as 1, while negative samples were coded as 0.

The Chi-square test was used to analyze the association of all examined factors as independent categorical variables with helminth and coccidian prevalence. Vari-

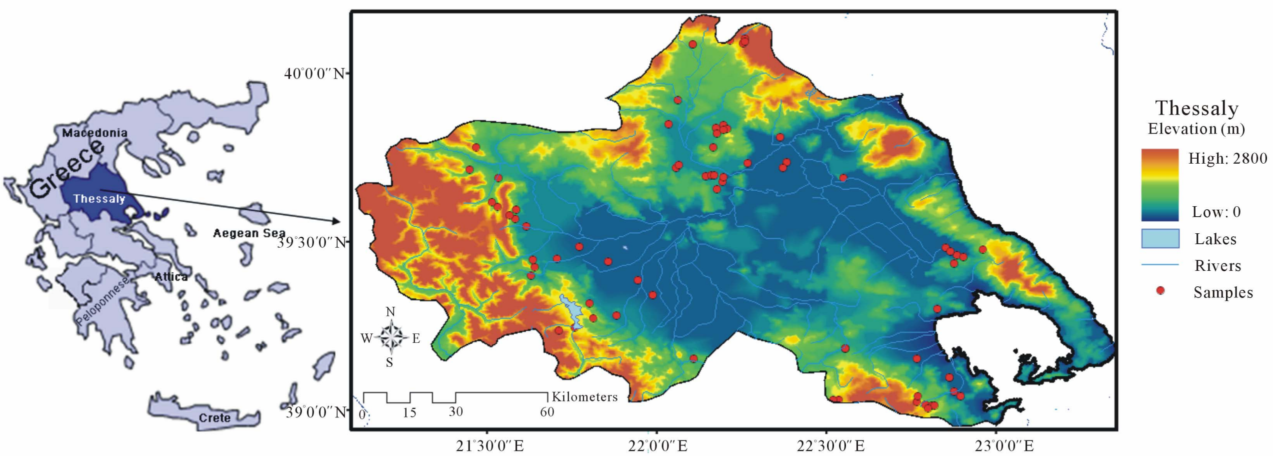

Figure 1. Geographical features of Thessaly, Greece.

ables significant at $p < 0.05$ were tested for multicolinearity and selected for inclusion in the multivariate logistic regression model. Overall fit of the logistic regression models was assessed using the Hosmer-Lemeshow goodness-of-fit statistics. Results are presented as adjusted odds ratios (OR) with 95% confidence intervals (95% CI). In all cases, the statistical significance was considered at 5% level. Missing observations were excluded from the analysis. All statistics were performed using the SPSS version 13.0 statistical package.

3. Results

A total of 31 organic farms (12% of all organic sheep and goat farms in Thessaly) and 38 neighboring conventional farms agreed to participate in the study. In total 557 fecal samples from sheep and goats belonging to 69 farms were collected. Helminth eggs were detected in 44 (7.9%) samples. Strongyle-type eggs were found in 19 (3.4%) samples, *Nematodirus* spp. eggs in 6 (1.1%) samples, *Trichuris* spp. eggs in 16 (2.9%) samples, *Fasciola hepatica* in 3 (0.5%) samples, and *Dicrocoelium dendriticum* in 1 (0.2%) sample. Coccidian oocysts were found in 36 (6.5%) samples (**Table 1**).

Univariate analysis of animal and farm-related factors (**Table 2**), grazing and farmer characteristics-related factors (**Table 3**) and environment-related factors (**Table 4**) showed that the rotation of grazing pastures, educational level of farmers, normalized difference vegetation index (NDVI) of farm location, and the elevation of farm location were associated with helminth infections. None of the examined factors was associated with coccidian infections (**Tables 5-7**). These significant factors were simultaneously analyzed in a logistic regression model to assess their relative contribution to helminth infections prevalence, while adjusting for their effects. The final logistic regression model showed that the educational level of farmers and the elevation of farm location were associated with helminth infections (**Table 8**). The risk of helminth infections in small ruminants was more than three-fold higher for farms owned or managed by farmers with a low educational level (elementary school or lower), compared with farms tended by farmers with a high level of education (middle school up to university) ($p < 0.05$). The risk of helminth infections was 0.36-fold lower for small ruminants in farms located in semi-mountainous areas compared to farms located in plain or mountainous areas ($p < 0.02$).

4. Discussion

The present study is the first epidemiological investigation of factors associated with GI parasitic infections of sheep and goats in Greece under a temperate Mediterranean climate. Therefore, the findings of this study can be generalized to other sheep and goat farms in areas with similar climatic conditions and management.

The spectrum of the GI parasites observed in sheep and goats in Greece comprised strongyles, *Nematodirus* spp., *Trichuris* spp. and coccidia at low to moderate prevalence. *Fasciola hepatica* and *Dicrocoelium dendriticum* were sporadically found, contrary to previous studies in Greece where the prevalence was higher on the basis of carcass inspection [14] and coproantigen or ELISA [11].

No significant risk factors were identified in the present study in regard to the presence of coccidian infections in sheep and goats. Nevertheless, studies under Mediterranean weather have shown a high prevalence of coccidian infections in lambs and goat kids [15,16] and adult animals [17]. Also, animal species was found to be another risk factor in a study in South Africa, where the mean monthly oocysts per gram of feces for goats was significantly higher than that for sheep [18].

Table 1. Prevalence (%) of gastrointestinal parasitic infections in small ruminants in Thessaly region, Greece.

	Prefecture									
	Thessaly (N = 557)		Trikala (N = 80)		Karditsa (N = 105)		Larissa (N = 202)		Magnisia (N = 170)	
Infection	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Helminths*	44	7.9 (5.9 - 10.5)	8	10 (4.4 - 18.8)	13	12.4 (6.8 - 20.2)	9	4.5 (2.1 - 8.3)	14	8.2 (4.6 - 13.4)
Strongyles	19	3.4 (2.1 - 5.4)	3	3.8 (0.8 - 10.6)	4	3.8 (1 - 9.5)	7	3.5 (1.4 - 7)	5	2.9 (1 - 6.7)
Nematodirus spp.	6	1.1 (0.4 - 2.5)	0	0 (0 - 4.5)	2	1.9 (0.2 - 6.7)	0	0 (0 - 1.8)	4	2.4 (0.6 - 5.9)
Trichuris spp.	16	2.9 (1.7 - 4.7)	4	5 (1.4 - 12.3)	5	4.8 (1.6 - 10.8)	0	0 (0 - 1.8)	7	4.1 (1.7 - 8.3)
Fasciola hepatica	3	0.5 (0.1 - 1.7)	2	2.5 (0.3 - 8.7)	1	1 (0 - 5.2)	0	0 (0 - 0.8)	0	0 (0 - 2.1)
Dicrocoelium dendriticum	1	0.2 (0 - 1.2)	0	0 (0 - 4.5)	1	1 (0 - 5.2)	0	0 (0 - 0.8)	0	0 (0 - 2.1)
Coccidia	36	6.5 (4.6 - 8.9)	3	3.8 (0.8 - 10.6)	5	4.8 (1.6 - 10.8)	14	6.9 (3.8 - 11.4)	14	8.2 (4.6 - 13.4)

*: Infection with all the investigated parasites except coccidian; CI: Confidence interval.

Table 2. Univariate analysis of various animal host and farm-related risk factors associated with helminth infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Species of small ruminant						0.44
Sheep	334	24	7.2	0.786	0.42 - 1.46	
Goat	223	20	9.0	1.00		
Herd size (number of animals)						0.10
<150	124	15	12.1	2.202	1.06 - 4.56	
150 - 300	107	9	8.4	1.47	0.63 - 3.40	
>300	289	17	5.9	1.00		
Unknown	37					
Farm type						0.69
Organic	269	20	7.4	0.88	0.47 - 1.63	
Conventional	288	24	8.3	1.00		
Anthelmintic treatment						0.27
No	116	12	10.3	1.47	0.73 - 2.96	
Yes	441	32	7.3	1.00		
Class of anthelmintic treatment						0.14
No anthelmintic	117	12	10.3	-	-	
Albendazole	388	26	6.7	-	-	
Other benzimidazoles	11	1	9.1	-	-	
Imidazothiazoles	23	5	21.7	-	-	
Salicylanilides	18	0	0	-	-	

Table 3. Univariate analysis of various grazing and farmer characteristics-related risk factors associated with helminth infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Grazing of animals						0.88
No	11	1	9.1	1.17	0.14 - 9.35	
Yes	546	43	7.9	1.00		
Grazing with other herds						0.25
Grazing alone/no contact	104	6	5.8	0.923	0.33 - 2.54	
Grazing with the same species	241	24	10	1.66	0.81 - 3.42	
Grazing with sheep, goats, cattle, pigs, and horses	193	12	6.2	1.00		
Unknown	19					
Rotation of grazing						0.04
No	455	40	8.8	3.90	0.92 - 16.4	
Yes	83	2	2.4	1.00		
Unknown	19					
Age of farmer (years)						0.06
18 - 44	227	12	5.3	0.52	0.26 - 1.03	
≥45	330	32	9.7	1.00		
Educational level of farmer						0.03
Basic level (elementary school or lower)	405	38	9.4	2.52	1.04 - 6.08	
High level (middle school up to university)	152	6	3.9	1.00		

Table 4. Univariate analysis of various environment-related risk factors associated with helminth infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Season						0.11
Autumn	292	18	6.2	0.60	0.32 - 1.12	
Winter	265	26	9.8	1.00		
Land surface temperature (C)						0.05
≤15	215	23	10.7	1.83	0.98 - 3.39	
>15	342	21	6.1	1.00		
NDVI						0.02
Low vegetation (0.40 - 0.50)	223	9	4.0	0.37	0.15 - 0.90	
Moderate vegetation (0.50 - 0.60)	216	23	10.6	1.03	0.50 - 2.20	
Dense vegetation (>0.60)	118	12	10.2	1.00		
Rain (mm)						0.95
450 - 600	148	11	7.4	0.88	0.39 - 1.96	
600 - 800	217	17	7.8	0.93	0.45 - 1.90	
>800	192	16	8.3	1.00		
Elevation of farm location (m)						0.01
Plain (1 - 200)	217	17	7.8	0.49	0.24 - 1.03	
Semi-mountainous (200 - 700)	230	11	4.8	0.29	0.13 - 0.66	
Mountainous (>700)	110	16	14.5	1.00		
Prefecture of farm location						0.09
Trikala	80	8	10	1.23	0.49 - 3.08	
Karditsa	105	13	12.4	1.57	0.70 - 3.49	
Larissa	202	9	4.5	0.52	0.21 - 1.23	
Magnisia	170	14	8.2	1.00		

Table 5. Univariate analysis of various animal host and farm-related risk factors associated with coccidian infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Species of small ruminant						0.88
Sheep	334	22	6.6	1.05	0.52 - 2.10	
Goat	223	14	6.3	1.00		
Herd size (number of animals)						0.14
150	124	5	4.0	0.67	0.242 - 1.86	
>150 - 300	107	11	10.3	1.83	0.829 - 4.05	
>300	289	17	5.9	1.00		
Unknown	37					
Farm type						0.63
Organic	269	16	5.9	0.84	0.43 - 1.67	
Conventional	288	20	6.9	1.00		
Anthelmintic treatment						0.52
No	116	6	5.2	0.74	0.30 - 1.84	
Yes	441	30	6.8	1.00		
Class of anthelmintic treatment						0.69
No anthelmintic	117	6	5.1	0.43	0.08 - 2.32	
Albendazole	388	26	6.7	0.57	0.12 - 2.63	
Other benzimidazoles	11	0	0.0	0	-	
Imidazothiazoles	23	2	8.7	0.79	0.97 - 6.08	
Salicylanilides	18	2	11.1	1.00		

Table 6. Univariate analysis of various grazing and farmer characteristics-related risk factors associated with coccidian infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Grazing of animals						0.15
No	11	2	18.2	3.34	0.69 - 16.1	
Yes	546	34	6.2	1.00		
Grazing with other herds						0.05
Grazing alone/no contact	104	3	2.9	0.28	0.83 - 1.04	
Grazing with the same species A	241	12	5.0	0.5	0.23 - 1.08	
Grazing with sheep, goats, cattle, pig and horses	193	18	9.3	1.00		
Unknown	19					
Rotation of grazing						0.58
No	455	29	6.4	1.34	0.46 - 3.93	
Yes	83	4	4.8	1.00		
Unknown	19					
Age of farmer (years)						0.64
18 - 44	227	16	7.0	1.17	0.59 - 2.32	
≥45	330	20	6.1	1.00		
Educational level of farmer						0.48
Basic level (elementary school or lower)	405	28	6.9	1.33	0.59 - 3	
High level (middle school up to university)	152	8	5.3	1.00		

Table 7. Univariate analysis of various environment-related risk factors associated with coccidian infections in small ruminants. Results are presented as odd ratios (OR) and 95% confidence intervals (CI).

Factor	N	n	Prevalence (%)	Crude OR	95% CI	p-value
Season						0.09
Autumn	292	14	4.8	0.55	0.27 - 1.12	
Winter	265	22	8.3	1.00		
Land surface temperature (C)						0.69
≤15	215	15	7.0	1.14	0.57 - 2.27	
>15	342	21	6.1	1.00		
NDVI						0.88
Low vegetation (0.40 - 0.50)	223	13	5.8	0.85	0.34 - 2.11	
Moderate vegetation (0.50 - 0.60)	216	15	6.9	1.02	0.42 - 2.49	
Dense vegetation (>0.60)	118	8	6.8	1.00		
Rain (mm)						0.47
450 - 600	148	11	7.4	1.63	0.65 - 4.04	
600 - 800	217	16	7.4	1.62	0.69 - 3.75	
>800	192	9	4.7	1.00		
Elevation of farm location (m)						0.80
Plain (1 - 200)	217	15	6.9	0.94	0.38 - 2.30	
Semi-mountainous (200 - 700)	230	13	5.7	0.76	0.30 - 1.90	
Mountainous (>700)	110	8	7.3	1.00		
Prefecture of farm location						0.48
Trikala	80	3	3.8	0.43	0.12 - 1.55	
Karditsa	105	5	4.8	0.55	0.19 - 1.59	
Larissa	202	14	6.9	0.83	0.38 - 1.79	
Magnisia	170	14	8.2	1.00		

Table 8. Logistic regression analysis of risk factors associated with helminth infections in small ruminants (final model, including both farm and environment-related factors).

Factor	N	n	Prevalence (%)	Adjusted OR	95% CI	p-value
Rotation of grazing						
No	455	40	8.8	2.07	0.44 - 9.0	0.36
Yes	83	2	2.4	1.00		
Unknown	19					
Educational level of farmer						
Basic level (elementary school or lower)	405	38	9.4	3.13	1.13 - 8.62	0.02
High level (middle school up to university)	152	6	3.9	1.00		
NDVI						
Low vegetation (0.40 - 0.50)	223	9	4.0	0.39	0.13 - 1.10	0.07
Moderate vegetation(0.50 - 0.60)	216	23	10.6	1.02	0.43 - 2.41	0.95
Dense vegetation (>0.60)	118	12	10.2	1.00		
Elevation of farm location (m)						
Plain (1 - 200)	217	17	7.8	0.90	0.38 - 2.15	0.82
Semi-mountainous (200 - 700)	230	11	4.8	0.36	0.15 - 0.86	0.02
Mountainous (>700)	110	16	14.5	1.00		

In the present study, animal species was not found to be a significant risk factor in regard to the presence of GI helminths, but in previous studies in Gambia [19] and Pakistan [20] it was found that goats carried significantly lower worm burdens than sheep.

Average NDVI of farm location for 12 months before sampling, which has been used as an indicator of regional thermal-moisture regimes, was the most significant environmental risk factor for GI helminth infections. NDVI values integrate a number of different environmental factors (land cover, temperature, rainfall, vapor pressure, etc.) into a single variable and thus simplify analysis [21]. In Sub-Saharan Africa, after adjusting for distance from the equator, low NDVI values are predictive of an increase in cryptosporidiosis in the following month [22]. On the contrary, in the present study, NDVI was not found to be a risk factor concerning coccidian infections.

The variables introduced into the statistical model that concern the age or the educational level of farmers had also been investigated by other authors in Italy [23] and in Kenya [24]. The educational level of farmer was an important risk factor of GI helminth infections in the present study. This finding is in agreement with reports from other investigators in Kenya [24]. It is known that a large part of the variation in the performance of livestock production is caused by the quality of the management-stockpeople team [25]. The low education level of farmers may be associated with a low degree of stockmanship and with a higher number of at-risk activities, such as the careless manipulation of offal, carcass, feces, water or feed in the farm, which favor the transmission of certain parasites [26].

Elevation of farm location was found in the present study to be a risk factor for GI helminth infections in sheep and goats. Farms located on high elevation had a higher prevalence of GI helminth infections. Apparently, high elevation areas have favorable climatic conditions for the development of the free-living stages of the GI nematodes. Elevation has also been found to be a risk factor for the presence of *F. hepatica* in sheep and goats in Greece where a significant correlation was detected between coproantigens of *F. hepatica* and elevation [11].

In conclusion, the present study provides the first epidemiological investigation of risk factors associated with GI helminth infections of sheep and goats in Greece. The emerging profile of sheep and goats at high risk for GI helminth infections in Greece is that of animals belonging to farms where the educational level of the farmer is low and the farm is located in a mountainous area with moderate vegetation. Therefore, increasing farmer awareness for worm control measures could be achieved through education of the farmers. In addition, these findings can be used to target high risk farms with appropriate control measure for GI helminth parasites of sheep and goats in Greece and other areas with similar climatic conditions.

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