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Seroprevalence and risk factors associated with zoonotic parasitic infections in small ruminants in the Greek temperate environment

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ABSTRACT

A cross-sectional serological study was carried out to screen the sheep and goat population of Thessaly, Greece for evidence of infection with *Toxoplasma*, *Toxocara*, *Leishmania*, and *Echinococcus* and to determine the risk factors related to herd characteristics, herd management practices, farmer status, and the bioclimatic variables associated with these zoonotic parasitic infections. A total of 540 sheep and goat serum samples were examined. The seroprevalence of infection in all examined animals was 24.5% for *Toxoplasma*, 32% for *Toxocara*, 0% for *Leishmania* and 85.9% for *Echinococcus*. The final logistic regression model showed that the species of small ruminant, herd size, anthelmintic treatment, class of anthelmintic treatment, grazing with other herds, educational level of farmer, elevation of farm location, and generalized land cover were associated with *Toxoplasma gondii* infections, while the species of small ruminant, farm type, anthelmintic treatment, class of anthelmintic treatment, rotation of grazing, age of farmer, elevation of farm location, and generalized land cover were associated with *Toxocara canis* infections. Antibodies to *T. gondii* were detected in 102 (28.3%) of 360 sheep and in 30 (16.8%) of 179 goats. Animals in small flocks (150–300 animals) had an approximately 0.42-fold lower risk of having positive cases of *T. gondii* among animals compared with large flocks (>300 animals). Antibodies to *T. canis* were found in 155 (42.9%) of 361 sheep and 18 (10.1%) of 179 goats. The later finding constitutes the first report of seropositive goats to *Toxocara*. The risk of positivity for *T. canis* was 7.71-fold higher in sheep than in goats. Geographically, animals from plain areas had 2.9 and 2.01-fold higher risk of having positive cases of *T. gondii* and *T. canis* respectively. The significant bioclimatic variables ($p < 0.05$) associated with the occurrence locations of *T. gondii* infection were related to higher temperature, lower precipitation, and lower elevation compared to the absence locations of *T. gondii*. The significant bioclimatic variables ($p < 0.05$) associated with occurrence locations of *T. canis* infection were related to lower temperature and higher precipitation compared to absence locations of *T. canis*. These findings are useful to formulate appropriate control strategies for zoonotic parasites of sheep and goats in Greece and other areas with similar climatic conditions.

1. Introduction

Toxoplasma, *Toxocara*, *Leishmania*, and *Echinococcus* are important zoonotic parasites of farm animals and wildlife with serious consequences to public health and livestock industry. In Europe, high seroprevalence of *Toxoplasma gondii* in sheep has been found in Switzerland (61.6%) [1] and Finland (24.6%) [2]. *Toxoplasma* has also been detected in pigs and wild animals in Europe [3–5]. In Greece, cases of *Toxoplasma* infections have been documented in humans [6], equids [7], dogs [8], pigeons [9], as well as sheep and goats [10–12]. *Toxocara canis* is an intestinal nematode affecting dogs whose larvae can infect humans. Specific antibodies to *T. canis* have been detected in sheep in UK [13] and Brazil [14]. In Greece, sporadic cases of toxocarosis have been reported in humans [15,16], while the infection in dog is very common [8,17]. *Leishmania infantum* is the cause of zoonotic visceral leishmaniosis in humans in the Old and New Worlds, with the dog as the main domestic reservoir [18]. In Greece, the prevalence of infection in dogs varies depending on the area, between 0.7% and 48.7% [19]. A low frequency of *L. infantum* antibodies in humans has been found in Greece [20] in agreement with reports from other Mediterranean countries where visceral leishmaniosis is a rare disease and only sporadic cases have been described [21]. Lately, cases of ruminant animals harboring *Leishmania* have been reported in Nepal [22,23],

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in Switzerland [24], and in Bangladesh [25] indicating the need for further investigation of ruminants' possible role in the transmission of zoonotic visceral leishmaniosis. The larval cystic stage of *Echinococcus granulosus* is the cause of hydatidosis in humans and herbivore farm animals. Hydatidosis is an increasing public health and socio-economic concern due to its considerable morbidity rates that give rise to high economic losses both in the public health sector and the livestock industry [26]. Published data suggest that the prevalence of cystic hydatidosis in the Mediterranean region is rather high [26,27]. In Greece, hydatidosis is a serious problem for both the livestock industry and the public health. Surveillance in livestock species has documented a high prevalence in sheep (30.4%) and goats (14.7%) [28]. The number of human cases in 2000 was reported to be 0.28 per 100,000 inhabitants [29].

In the present study, a cross-sectional serological survey was carried out to (i) screen the sheep and goat population of Thessaly, Greece for evidence of infection with *Toxoplasma*, *Toxocara*, *Leishmania*, and *Echinococcus* and (ii) determine the risk factors related to herd characteristics, herd management practices, farmer status, and the bioclimatic variables associated with these zoonotic parasitic infections.

2. Materials and methods

2.1. Sampled areas

The region of Thessaly covers an area of 14037 km^2 and is located in Central Greece, centered at latitude of 39°30′0″N and longitude of 22°0′0″E. This region 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.

2.2. Sample and data collection

Serum samples were collected from clinically healthy and randomly selected sheep and goats in organic and neighboring conventional farms registered with the Hellenic Ministry of Rural Development and Food, according to the latest available census (2005) in the region of Thessaly, Greece. No particular criteria were followed during sampling in the two types of farms, the emphasis being on animals rather than on farms. Also, both sheep and goats were regarded as a single unit in sample size determination, due to the fact that they are both susceptible and serve as reservoir to the zoonotic parasites under investigation. Since no idea of the actual prevalence of the infections was known, the sample size of both species combined was planned to be well above than required ($n = 384$) for the expected parasite prevalence of 50% at the absolute precision of 5% for a 95% confidence interval [30]. Farms whose owners agreed to participate in the study were visited once and were equally distributed by autumn and winter seasons. Serum samples were stored at −20 °C until analyzed. Data on herd characteristics, herd 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.

2.3. Source of environmental data

Climate data for farm locations were obtained by WorldClim version 1.4 climate data [31] available on the WorldClim website (http://www.worldclim.org). WorldClim provides monthly data as 50-year means for precipitation, minimum temperature, and maximum temperature. These data are further processed into 19 bioclimatic variables that serve as indicators of the climate regime of the area (Table 1). WorldClim website also provides elevation data processed from NASA Shuttle Radar Topography Mission (SRTM) data to have the same projection and resolution as the other WorldClim layers. The WorldClim bioclimatic variables and elevation data with an approximate resolution of 1 km were used for this study.

Land cover data were obtained from the U.S. Geological Survey's (USGS) Global Land Cover Characteristics Database version 2 Global (http://edcsns17.cr.usgs.gov/glcc/). The USGS website provides data in several classification schemes; the global ecosystems land cover classification was selected for this study. This land cover classification uses 100 classes, 10 of which corresponded to the collection localities of the zoonotic parasites under study. To simplify the statistical analysis, however, the original 10 classes were combined into 3 generalized classes, as listed in the second column of Table 2.

2.4. Sera preparation and ELISA test

2.4.1. Sera

Sera were obtained by centrifugation of clotted blood and stored at −20 °C until used. ELISA was performed on serum samples for the detection of specific IgG antibodies against *T. gondii*, *T. canis*, *L. infantum*, and *E. granulosus*.

2.4.2. Antigens

The antigens used in the assay were homemade [7,32]. *T. gondii* was isolated from the peritoneal cavity of experimentally infected BALB/c mice. Purified *Toxoplasma* tachyzoites were adjusted to a concentration of 2×10^6 organisms/ml in phosphate-buffered saline (PBS, pH 7.2) and soluble protein extracts were obtained by freezing/thawing cycles (liquid nitrogen/25 °C), of the parasites. The extracts were clarified by centrifugation at 3500 ×*g* for 15 min and the supernatant was centrifuged at 34,000 ×*g* for 2 h at 4 °C. The final supernatant was collected and used as soluble antigen in ELISA. *T. canis* antigen was prepared by grinding whole adult roundworms, collected from infected dogs, in an adequate grinding bowl, with saline. The liquid material obtained was centrifuged (15,000 ×*g* for 15 min at 4 °C) and the supernatant was sonicated at 50 kHz for 2 min in an ice bath. The material was then

Table 1
List of WorldClim bioclimatic variables [31] used in the comparison of the bioclimatic variables between absence and occurrence locations of parasitic infections.

Bioclimatic variable	Description
BIO1	Annual mean temperature
BIO2	Mean diurnal range (mean of monthly(max temp − min temp))
BIO3	Isothermality (BIO2 / BIO7) (∗100)
BIO4	Temperature seasonality (standard deviation ∗ 100)
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7	Temperature annual range (BIO5 − BIO6)
BIO8	Mean temperature of wettest quarter
BIO9	Mean temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter