



## Research paper

## Regression models to assess the risk factors of canine gastrointestinal parasitism

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## ABSTRACT

Gastrointestinal parasites, protozoa and helminths, remain a common important finding in dogs presented to veterinary medical practices. Moreover, dogs may play an active role in the transmission of enteric parasites to humans, given their cohabitation, making canine gastrointestinal parasitism an issue of major concern for public health. To get an overview of the current state of canine gastrointestinal parasites' prevalence in Greece, a total of 1036 faecal samples were collected from dogs either presented in veterinary clinics or in animal shelters. Samples were examined by a combined sedimentation–flotation technique. Possible risk factors (age, ownership status, co-existence with other animals, coinfection with other parasites) were assessed using binary regression models for each one of the most prevalent parasites. The overall gastrointestinal parasite prevalence in dogs' faecal samples was 39%. A total of 11 intestinal parasitic species were detected and up to five different parasites were isolated in the same faecal sample. *Toxocara canis* was the most prevalent parasite in the studied population, followed by *Isoospora* spp., *Giardia* spp., *Uncinaria* spp., *Trichuris vulpis*, *Ancylostoma* spp., *Toxascaris leonina*, and *Dipylidium caninum*. Additionally, other genera (*Taenia*, *Capillaria* and *Angiostrongylus*) were also detected in very low percentages. Young, stray, living without other dogs, coinfecting with *T. vulpis* or *T. canis* and free of *Isoospora* spp. dogs were more likely to be infected by *Giardia* spp. Likewise, young, stray, coinfecting with *T. vulpis* or *T. canis* but free of *Giardia* spp. dogs were more likely to be infected by *Isoospora* spp. *T. canis* infections were more common in dogs coinfecting with *Isoospora* spp., *Giardia* spp., *D. caninum*, *Taenia* spp., *T. vulpis* and *T. leonina*. Finally, *T. vulpis* more often infected older dogs and dogs coinfecting with *Isoospora* spp., *Giardia* spp. and *T. canis*. Consequently, the prevalence of parasitized dogs was high and the diversity of parasites found was notable, which calls for a greater awareness among veterinarians and pet owners. The proposed prediction models should be taken under consideration in diagnostic approach of clinical cases, as well as in planning sustainable antiparasitic strategies.

## 1. Introduction

Dogs are in close relationship with humans offering several benefits, including guarding, hunting and companion (Mateus et al., 2014). They can be exposed to a varying degree of disease risk, especially parasitism, depending on their lifestyle. Parasites, particularly the gastrointestinal ones, such as *Isoospora* (syn. *Cystoisospora*) spp. and *Giardia* spp., may pose a serious threat to dogs' health and welfare, causing severe clinical signs and even death, especially in puppies (Hall and German, 2005; Ryan and Caccio, 2013). Additionally, some of them, such as *Toxocara canis*, *Echinococcus granulosus*, *Giardia* spp. and *Ancylostoma* spp., may have public health significance (Martinez-Moreno

et al., 2007; Claerebout et al., 2009).

Monitoring the active regional prevalence of canine intestinal parasites must be an ongoing task and constitutes an important first step towards effective surveillance and prevention (Traub et al., 2005). These data, when intergraded in a prediction model, can produce a useful rapid tool to optimize control strategies. Moreover, dog owner's awareness of the risks involved is another crucial aspect for reducing the zoonotic potential of pet infections (Claerebout et al., 2009; Riggio et al., 2013).

Surveys for dog parasitism have been conducted throughout Europe, in countries such as Italy (Rinaldi et al., 2006; Riggio et al., 2013; Simonato et al., 2015), Portugal (Neves et al., 2014), Germany

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(Barutzki and Schaper, 2011; Becker et al., 2012), Belgium (Claerebout et al., 2009), Spain (Martinez-Moreno et al., 2007), Hungary (Fok et al., 2001), Lithuania (Bruzinskaite et al., 2009) and the Czech Republic (Dubna et al., 2007). These studies have demonstrated that intestinal parasites are common in dogs with reported prevalence estimates ranging significantly among different countries. In Greece, only a very limited number of studies deals with distribution and risk factors of canine gastrointestinal parasitism (Haralabidis et al., 1988; Papazahariadou et al., 2007), even though this part of Europe is of great epidemiological importance given the constant flow of pets associated with tourism (Otranto, 2015; Maia and Cardoso, 2015).

The aim of this study was to investigate the current epizootiological profile of dog parasitism in Greece in view of creating a prediction model to assess the likelihood of infection with the most commonly identified parasites.

## 2. Materials and methods

### 2.1. Faecal sample collection

A total of 1036 dogs were included in the study during the period from September 2015 to May 2016. The sampled dogs were presented to local animal shelters and veterinary practices for either clinical examination, preventive veterinary practices (e.g. vaccination) or without specified reason. From each individual dog, a faecal sample was collected either immediately after spontaneous elimination or fresh from kennel grounds avoiding environmental contamination. Information regarding age, gender, ownership status, breed, coexistence with others animals and lifestyle were collected for each animal sampled. Samples were placed individually in plastic containers, labeled with consecutive numbers, stored at 2–6 °C, transferred to the Laboratory of Parasitology and Parasitic Diseases of the School of Veterinary Medicine in Thessaloniki and processed within 48 h.

### 2.2. Study area and dog population

Dogs' faecal samples were collected from both the mainland, namely Macedonia (30.9%), Central Greece (27.6%), Thrace (10.8%), Thessaly (7.9%), Peloponnese (5.8%), Epirus (5.5%) and the islands (11.5%) of Greece. Regarding ownership, samples were collected from owned (60.8%,  $n = 630$  dogs) and from stray dogs (39.1%,  $n = 406$  dogs). Gender of dogs was almost evenly distributed with 491 (47.4%) male and 545 (52.6%) female animals. The majority of dogs were adult i.e. > 1 year (90.3%,  $n = 936$  dogs), while young dogs represented 9.7% of the studied population ( $n = 100$  dogs). Eight hundred seventy of the dogs in the study were crossbred (83.9%), while the rest 166 dogs (16.0%) were purebred from different breeds. Regarding companionship and lifestyle, most of the dogs were living at the same place with other dogs (62.5%,  $n = 648$  dogs) rather than alone (37.5%,  $n = 388$  dogs) and outdoors (91%,  $n = 942$  dogs) rather than indoors (9.0%,  $n = 94$  dogs). None of the examined animals received any anthelmintic treatment minimum 3 months before sampling.

### 2.3. Coprological methods used

Initially, each sample was macroscopically examined to detect the possible presence of cestode proglottids and adult nematodes. Thereafter, a combined sedimentation–flotation (ZnSO<sub>4</sub> 33.2%) technique was used for further examination of the samples (Bauer 2006; Foreyt, 2001). Identification of parasitic elements was based on morphological characteristics (Thienpont et al., 1986; Zajac and Conboy, 2012). A dog was considered infected if at least one parasitic element (cyst, oocyst, egg, proglottid or larva) was observed.

### 2.4. Data handling – statistical analyses

All data were recorded in a specially designed Microsoft Excel spreadsheet, appropriately developed for the subsequent statistical analyses. Four binary logistic regression models were used in order to test hypotheses about the possible relationship between the most significant predictors and the likelihood that a dog is infected by the most prevalent parasites, namely *Giardia* spp., *Isoospora* spp., *T. canis* and *Trichuris vulpis*. A stepwise procedure was followed and only predictors with significant effects on the parasitic infections were used for the final models. The predictors for the likelihood of *Giardia* spp. infection were age, ownership status, companionship and infection by *Isoospora* spp., *T. canis* and *T. vulpis* as described in Model 1:

$$(\text{Model 1}) Y_G = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6$$

where,

$Y_G$  = the probability of a dog being infected by *Giardia* spp.,  $\beta_1$  to  $\beta_6$  the regression coefficients of age ( $X_1$ ,  $0 \leq 1$  year,  $1 \geq 1$  year), ownership status ( $X_2$ ,  $0 =$  with owner,  $1 =$  without owner), companionship ( $X_3$ ,  $0 =$  kept with other animal,  $1 =$  alone), and *Isoospora* spp., *T. vulpis* and *T. canis* infection status ( $X_4$ ,  $X_5$ ,  $X_6$ , respectively, with 0 indicating the absence and 1 the presence of infection).

A similar model was developed for the estimation of the likelihood of *Isoospora* spp. infection; in this model, *Giardia* spp. infection was added into the model, whereas, ownership status and *Isoospora* spp. infection status were excluded from it. The probability of *T. canis* infection was estimated using as predictors the regression coefficients of the dogs' infection status regarding other parasites, namely *Giardia* spp., *Isoospora* spp., *T. vulpis*, *Toxascaris leonina*, *Taenia* spp. and *Dipylidium caninum*. *T. vulpis* infection probability was calculated forcing into the model the regression coefficient of age and the coefficients of *Giardia* spp., *Isoospora* spp. and *T. canis* infection status.

Statistical significance of individual predictors was tested using the Wald  $\chi^2$  statistic of their regression coefficients ( $\beta$ s). Goodness-of-fit for each individual model was assessed using the Hosmer-Lemeshow (H-L) test, as well as Cox and Snell  $R^2$  and Nagelkerke  $R^2$  indices. In order to assess the overall ability of the models to correctly predict parasitic infections, the Receiver Operating Characteristic (ROC) curves analyses were used and the areas under the ROC curves (AUC, c-statistic) were estimated.

For the validation of the models, a 80-20 cross-validation analysis was adopted, with a randomly selected training sample (the sample used to built the model) including 80% of the studied cases and a holdout sample including the rest 20% of them; during validation of the model the holdout sample was classified using the coefficients, derived by the model built using the training sample. The classification accuracy of the holdout sample was used to estimate the effectiveness of the model performance for the studied population of dogs; a classification accuracy within 10% of the training sample was considered to evidence the utility of the model. Moreover, the model was considered efficient when the significance of the predictors' coefficients in the full-data model matched with the coefficients' significance in the training sample model.

## 3. Results

### 3.1. Epidemiology of gastrointestinal parasites in the studied dog population

Overall, 39.1% (405/1036) of the studied dogs were found to excrete at least one parasitic element. Among positive animals ( $n = 405$ ), 236 dogs (58.3%) were infected by only one parasite genera, while 123 (30.4%), 48 (11.9%), 11 (2.7%) and 3 (0.7%) dogs were found to be infected by two, three, four and five different parasite genera, respectively.

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