Risk factors and geospatial modelling for the presence of *Fasciola hepatica* infection in sheep and goat farms in the Greek temperate Mediterranean environment

V. KANTZOURA[1], M. K. KOUAM[1], N. DEMIRIS[2], H. FEIDAS[3] *and* G. THEODOROPOULOS[1]*

[1] *Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science and Hydrobiology, Agricultural University of Athens, 75 Iera Odos, Votanikos, Athens 11855, Greece*
[2] *Department of Animal Breeding and Husbandry, Faculty of Animal Science and Hydrobiology, Agricultural University of Athens, 75 Iera Odos St., Votanikos, Athens 11855, Greece*
[3] *Department of Meteorology and Climatology, School of Geology, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*

(*Received 22 December 2010; revised 2 February 2011; accepted 14 February 2011*)

SUMMARY

Risk factors related to herd and farmer status, farm and pasture management, and environmental factors derived by satellite data were examined for their association with the prevalence of *F. hepatica* in sheep and goat farms in Thessaly, Greece. Twelve farms (16·2%) and 58 farms (78·4%) of 74 had evidence of infection using coproantigen and serology respectively. The average normalized difference vegetation index (NDVI) of farm location for 12 months before sampling was the most significant environmental risk factor for *F. hepatica* infection based on high seropositivity. The risk of infection increased by 1% when the value of NDVI increased by 0·01 degree. A geospatial map was constructed to show the relative risk (RR) of *Fasciola* infection in sheep and goat farms in Thessaly. In addition, geospatial maps of the model-based predicted RR for the presence of *Fasciola* infection in farms in Thessaly and the entire area of Greece were constructed from the developed model based on NDVI. In conclusion, this study demonstrated that Thessaly should be regarded as an endemic region for *Fasciola* infection and it represents the first prediction model of *Fasciola* infection in small ruminants in the Mediterranean basin.

Key words: *Fasciola hepatica*, sheep, goats, coproantigen, serology, risk factors, GIS, risk map, Greece.

INTRODUCTION

The common liver fluke *Fasciola hepatica* (Linnaeus, 1758) (Trematoda: Fasciolidae) has a worldwide distribution (Mas-Coma, 2004) and infects several species of mammals, particularly cattle and sheep. Intermediate hosts are freshwater snail species of the family Lymnaeidae (Mas-Coma and Bargues, 1997).

Infected animals show lowered weight gain, anaemia, reduced fertility, reduced milk production and lowered feed conversion efficiency (Hillyer, 2005). Despite the substantial economic losses caused by *F. hepatica*, estimated at US$ 2 billion per year worldwide (Spithill and Dalton, 1998) and the cosmopolitan distribution of this parasite, little attention has been given to the study of risk factors of fasciolosis in sheep and goats. A number of epidemiological studies in Europe, Africa, Asia and Australia have identified several risk factors of fasciolosis in cattle caused by *F. gigantica* and/or *F. hepatica* (Tum *et al*. 2004; Durr *et al*. 2005; McCann *et al*. 2010; Bennema *et al*. 2011). The actual risk of infection is influenced by the number and distribution of animals, the presence of infected snails, and grazing management which allow animals to access herbage or water containing metacercariae (Tum *et al*. 2004). These factors act largely on the hosts of the parasite rather than directly on the parasite itself. If there is no clear indication of the source of infection, careful study of risk factors possibly including environmental and herd management practices, should pinpoint the source of infection and can contribute to effective control programmes (Roberts and Suhardono, 1996).

Geographic Information System (GIS) technologies are being used increasingly to study the spatial and temporal patterns of *Fasciola* infection (McCann *et al*. 2010). GIS can be used to complement conventional ecological monitoring and modelling techniques and provide a means to portray complex relationships in the ecology of disease (Yilma and Malone, 1998). Monitoring the spatial distribution of economically important infections such as *F. hepatica* using GIS technologies can facilitate the study of the presence and location of high risk areas, thus providing possibilities for regionally adapted control measures (Beck *et al*. 2000; Bennema *et al*. 2009).

---

* Corresponding author: Department of Anatomy and Physiology of Farm Animals, Faculty of Animal Science and Hydrobiology, Agricultural University of Athens, 75 Iera Odos, Votanikos, Athens 11855, Greece. Tel: +30-201-5294387. Fax: +30-201-5294388. E-mail: gtheo@aua.gr

Parasitology (2011), **138**, 926–938.
doi:10.1017/S0031182011000436

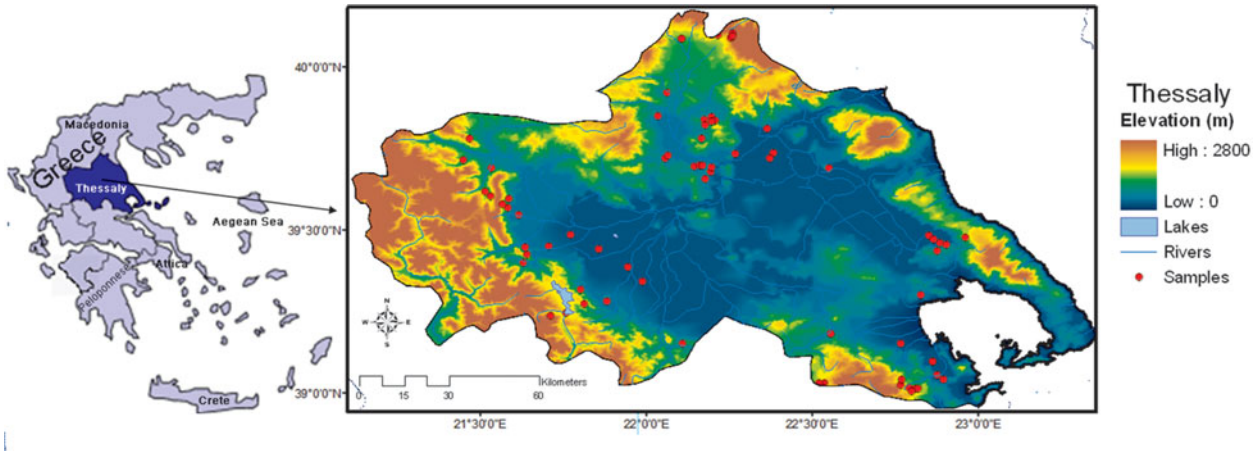

Fig. 1. Geographical features of Thessaly, Greece.

No previous study in Greece has been performed on risk factors for *Fasciola* infection in sheep and goats. In Greece, a major sheep and goat producing country, the knowledge of the epidemiology of fasciolosis is still limited with only a small number of studies documenting the occurrence of *F. hepatica* in sheep and snails (Antoniou *et al.* 1997; Theodoropoulos *et al.* 2002). The objectives of the present study were (i) to investigate the prevalence of *F. hepatica* in sheep and goat farms in the region of Thessaly, Greece using coproantigen and serology methods, (ii) to indentify the risk factors associated with *Fasciola* infection in sheep and goat farms, (iii) to model the risk of *F. hepatica* infection in sheep and goat farms using GIS technologies, (iv) to predict the distribution range of *F. hepatica* infection in sheep and goat farms on the basis of high seropositivity and (v) to extend this prediction to the entire area of Greece as well as the Mediterranean region.

MATERIALS AND METHODS

Study area

The region of Thessaly covers an area of 14 037 km^2 and is located in Central Greece, centred at a latitude of 39°30′0″N and a longitude of 22°0′0″E (Fig. 1). 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. There are droughts during the summer months. Mean annual precipitation over the whole Thessaly region is about 700 mm and varies from about 400 mm at the central plain area to more than 1850 mm in the western mountain peaks (Loukas *et al.* 2007).

Sample and data collection

Faecal and serum samples were collected from clinically healthy and randomly selected sheep and goats in organic and neighbouring 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. 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. Faecal and serum samples were stored at −20 °C until analysed.

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 20 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.

Source of environmental data and modelling

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 were 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 (Malone *et al.* 2001). The environmental data were recorded as monthly means for 12 months before the day of sampling for each examined farm.

The integration of satellite data into epidemiological research enhances the spatio-temporal resolution of climatological data, in particular in mountainous regions where weather stations and ground surveys are unavailable or sparse. MODIS data, as they deliver daily 2 global coverages at 250 m–1000 m resolution, they are most useful to support epidemiological studies. The LST algorithm needs a pair of daytime and nighttime radiance data in 7 thermal infrared bands, atmospheric temperature and water vapour in the MODIS atmospheric product. The day/night LST product is generated by the generalized split-window LST algorithm (Wan and Dozier, 1996). The monthly LST MOD11C3 product provides monthly composited and averaged temperature values at 0·05 degree latitude/longitude grids. This product is ready for use in science applications. It should be pointed out that the MODIS LST product based on thermal infrared data is only available in clear sky conditions.

Monthly NDVI is a composite of the NDVI daily values from cloud-free observations in the month from the MODIS blue, red, and near-infrared reflectances data. Low sampling from satellites due to cloud cover and other reasons is not a major problem for studies that require long-term LST and NDVI data sets. Neteler (2004) validated the usability of MODIS/Terra data in epidemiological studies in Italy as an enhancement of data availability, by investigating the monthly mean temperatures of selected meteorological stations and the related MODIS data at the same coordinates. Both datasets are matching surprisingly well except in the case of months with nearly continuous cloud cover with data availability less than 15%.

The 3B43 monthly rainfall data are derived by optimally merging a multi-satellite monthly product with rain gauge data (Huffman *et al*. 2007). A validation study conducted by Feidas (2010) demonstrated the excellent performance of the 3B43 product over Greece.

Geographical coordinates and elevation data of farm locations were obtained from the Digital Elevation Model (DEM), SRTM30 dataset for Greece with a resolution of 1 km 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 geographical features of Thessaly.

Coproantigen detection

The Bio-X bovine *Fasciola hepatica* ELISA test kit (Bio-X Company S.P.R.L., Belgium) was used on faecal samples according to the manufacturer's instructions. The plates were read in a 450 nm filter using an automatic plate reader (Infinite M200, Tecan). The calculation of the net optical density of each sample and positive control was done by subtracting the optical density reading of the negative control from each corresponding sample well. The limit of positivity for the antigen was 0·150. Any sample that yielded a difference in optical density that was greater than or equal to 0·150 was considered as positive.

Serology

The ELISA test was performed on serum samples as previously described (Salimi-Bejestani *et al*. 2005) using excretory secretory (E/S) antigen at 0·5 μg/ml to coat the plates. The sheep/goat serum samples were tested at 1:400 and the conjugate used was monoclonal anti-goat and sheep IgG conjugated to horse-radish peroxidase (Sigma-Aldrich, Germany) at a dilution of 1:10 000. In each well 100 μl of freshly prepared substrate TMB/HRP (3,3′, 5,5′-tetramethylbenzidine, hydrogen peroxide and proprietary catalysing and stabilizing agents (Uptima, Interchim, USA) were added and left for 20 min. The reaction was stopped by the addition of 100 μl of 0·5 M HCl per well. The results were expressed as an antibody index, using the following calculation: [(sample mean OD)/(positive pool mean OD] × 100. In each ELISA test, negative and positive controls were included and tested in quadruplicate. The cut-off value was the 25 percent positivity (pp) with sensitivity and specificity 77% and 93% for sheep and 86% and 100% for goats respectively. Seropositivity was divided into 2 categories: high (> 51pp) and low (25–50pp) seropositivity.

Statistical analysis

The basic model was a trivariate logistic regression with correlated random effects. Suppose that y_i^C, y^H and y_i^L denote the numbers of positive samples on farm i due to coproantigen and high and low seropositivity respectively. We assumed that y_i^C, y_i^H and y_i^L follow a binomial distribution, where n_i^C, n_i^H and n_i^L are the numbers of infected farms while ρ_i^C, ρ_i^H and ρ_i^L denote the probability of a farm being infected as determined by the coproantigen as well as by the high and low seropositivity, respectively. Therefore, we have:

$$y_i^C \sim \mathrm{Bin}(\rho_i^C, n_i^C)$$
$$y_i^H \sim \mathrm{Bin}(\rho_i^H, n_i^H)$$
$$y_i^L \sim \mathrm{Bin}(\rho_i^L, n_i^L)$$

Note that ρ_i^C, ρ_i^H and ρ_i^L were treated as random variables, the logit transformation of which is being linearly associated to the explanatory variables x as follows:

$$\mathrm{logit}(\rho_i^C) = b_0 + b_1 x_{i1} + b_2 x_{i2} + \ldots + b_m x_{im} + \varepsilon_i^C$$
$$\mathrm{logit}(\rho_i^H) = c_0 + c_1 x_{i1} + c_2 x_{i2} + \ldots + c_m x_{im} + \varepsilon_i^H$$

and

$$\mathrm{logit}(\rho_i^L) = d_0 + d_1 x_{i1} + d_2 x_{i2} + \ldots + d_m x_{im} + \varepsilon_i^L$$

where m is the number of the explanatory variables.

In our study we had 26 factors ($m = 26$) under consideration. On the above equations, x_{i1} to x_{i26} denote the studied risk factors, while ε^C corresponds to random effects for coproantigen and ε^H, ε^L corresponds to random effects for high and low seropositivity. We included 1 random effect per farm. In addition, we allowed ε^C, ε^H and ε^L to be correlated in order to capture the potential dependence between corpoantigen and seropositivity (High and Low) prevalence. We also allowed for the high and low seropositivity to be correlated. In summary, the random effects were assumed to follow a trivariate normal distribution as follows:

$$\begin{pmatrix} \varepsilon_i^C \\ \varepsilon_i^H \\ \varepsilon_i^L \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}, \Sigma_2\right)$$

$$\text{where } \Sigma_2 = \begin{pmatrix} \sigma_C^2 & \rho_{CH}\sigma_C\sigma_H & \rho_{CL}\sigma_C\sigma_L \\ \rho_{CH}\sigma_C\sigma_H & \sigma_H^2 & \rho_{HL}\sigma_H\sigma_L \\ \rho_{CL}\sigma_C\sigma_L & \rho_{HL}\sigma_H\sigma_L & \sigma_L^2 \end{pmatrix}$$

Thus, our model consists of 3 components, the different positivity indicators, namely coproantigen, high seropositivity, and low seropositivity. We used stepwise regression with a screening test ($P < 0{\cdot}2$) while statistical significance was considered at the 5% level. The analysis was carried out in the WinBugs software (Lunn *et al*. 2000).

Spatial cluster analysis

The spatial scan statistic implemented in SaTScan software (version 8.0) was used to investigate geographical clusters of infection. The concept of the spatial scan statistic is based on the generalization of a test probability (Turnbull *et al*. 1990; Kulldorff and Nagarwalla, 1995; Kulldorff, 1997). The spatial scan statistic uses a circular window of variable radius that moves across the map to represent potential geographical clusters. The radius of the cluster varies from zero up to a specified maximum value. By gradually changing the circle centre and radius, the window scans the geographical areas for potential localized clusters without incorporating prior assumptions about their size and location and noting the number of observed and expected observations inside the window at each location. The test of significance is based on the likelihood ratio test for which the window with the maximum likelihood is the most likely cluster (Kulldorff and Nagarwalla, 1995). The assessment of a cluster is done by comparing the number of cases (infection) within the circle with the number of expected cases under the assumption that cases are randomly distributed in the space. The P-value is obtained through Monte Carlo hypothesis testing (Dwass, 1957). The spatial scan statistic adjusts for spatial variations in the density of the population in the study area (Kulldorff, 1997). The population in the Poisson probability model may be the actual count from a census or covariate adjusting the expected counts from a statistical regression model while in the Bernoulli model. It is denoted as the total number of cases (positive samples) and controls (negative samples) in the study area. The detection of clusters in the present study was performed under the Bernoulli probability model using the maximum cluster size of 50% of the total population for *Fasciola* infection. Test-positive farms were considered as cases while test-negative farms were regarded as controls. The number of simulations for Monte Carlo testing was set to 9999. For each window of varying position and size, the SaTScan program tested the risk of *Fasciola* infection within and outside the window, with the null hypothesis of equal risk.

Risk maps

The spatial database GADM, version 1.0 (http://www.gadm.org/home) for country outlines and administrative subdivisions was used. The GIS software ARCGIS ver. 9.2 was used to display the sampled localities, the observed relative risk, and the predicted relative risk.

RESULTS

Prevalence and determination of risk factors for Fasciola hepatica *infection*

A total of 34 organic farms (13% of all organic sheep and goat farms in Thessaly) and 40 neighbouring conventional farms agreed to participate in the study. In total, 346 and 234 faecal samples as well as 499 and 372 serum samples were collected from sheep and goats respectively from 74 farms. Twelve farms (16·2%) and 58 farms (78·4%) of 74 were found infected using coproantigen and serology respectively (Table 1). A farm was considered as infected when at least 1 animal was found to be positive either on the basis of coproantigen or serology test.

According to the results of the statistical analysis, the correlation between the probability of a farm being infected as determined by the coproantigen (ρ_i^C) and high seropositivity ($\rho_i^{H)}$ was 0·97 (95% CI: 0·76 to 0·99) (Table 2). In contrast, the correlation between the probability of a farm being infected as determined by (a) the coproantigen (ρ_i^C) and low seropositivity (ρ_i^L) and (b) high (ρ_i^H) and low seropositivity (ρ_i^L) was lower and not statistically significant (at the 5% level), so the corresponding results are not presented. All the results of the statistical analysis and the risk factors as determined by coproantigen (component 1 of the model) and high seropositivity (component 2 of the model) are presented in Tables 3, 4, 5 and 6.

Table 1. Prevalence of *Fasciola hepatica* infection in farm and animal groups as determined by coproantigen and seropositivity

	Coproantigen		Seropositivity		High seropositivity	Low seropositivity
Group	*N**	*n*** (%)	*N*	*n* (%)	*n* (%)	*n* (%)
All farms	74	12 (16·2)	74	58 (78·4)	29 (39·2)	56 (75·7)
Sheep farms	40	8 (20)	40	34 (85)	22 (55)	34 (85)
Goat farms	34	4 (12)	34	24 (70·1)	7 (20·6)	22 (64·7)
All animals	580	48 (8·3)	871	295 (33·9)	89 (10·2)	206 (23·7)
Sheep	346	39 (11·3)	499	236 (47·3)	78 (15·6)	158 (31·7)
Goats	234	9 (3·8)	372	59 (15·9)	11 (3)	48 (12·9)

* *N*, Total number; ** *n*, Infected.

Table 2. Correlation between the probabilities of a farm being infected as determined by the coproantigen, high, and low seropositivity

Correlation	Mean	95% CI
Coproantigen/high seropositivity	0·97	(0·76, 0·99)
Coproantigen/low seropositivity	0·44	(−0·24, 0·87)
High seropositivity/low seropositivity	0·39	(−0·23, 0·87)

The risk of infection in the first component of the model was influenced by 13 factors associated with herd characteristics, farm and pasture management, by 1 factor associated with farmer characteristics, and by 4 environmental factors. The risk of infection in the second component of the model was influenced by 10 factors associated with herd characteristics, farm and pasture management, by all factors (2) associated with farmer characteristics, and by 2 environmental factors. The average NDVI of farm location for 12 months before sampling was the most significant environmental risk factor for *F. hepatica* infection in the second component of the model and the risk of infection increased by 1% when the value of NDVI increased by 0·01 degree.

Spatial distribution and geospatial modelling of Fasciola hepatica *infection*

NDVI as the main determined environmental risk factor in component 2 of the model was used for the construction of observed and predicted risk maps because NDVI values integrate a number of different environmental factors (land cover, temperature, rainfall, vapour pressure, etc.) into a single variable and thus simplifies analysis (Hay *et al*. 1997). The observed relative risk (RR) of *Fasciola* infection was calculated for each observed NDVI value in the examined farms while adjusting for all the other significant factors. The predicted RR of *Fasciola* infection was calculated for each NDVI value in the 0·25–0·81 range corresponding to the NDVI values in Thessaly not including urban areas. Hence, the prediction for the RR of *Fasciola* infection in Greece as a whole was extrapolated by also calculating the RR for each NDVI value in the 0·25–0·81 range, corresponding to the NDVI values in Greece not including urban areas. Thus, the map of RR of *Fasciola* infection for Thessaly and the maps of the model-based predicted RR for the presence of *Fasciola* infection in farms in Thessaly and the entire area of Greece were constructed.

The constructed model indicated that the areas of observed high RR of *Fasciola* infection were located in the western and south-eastern parts of Thessaly (Fig. 2A). The results of the spatial scan statistic analyses showed 1 most likely cluster ($P<0{\cdot}001$) of infected farms with *F. hepatica* in south eastern Thessaly and 2 secondary clusters in western and northern Thessaly (Fig. 2A and Table 7). The RR of the most likely cluster was 5·70.

The model-based prediction showed that the RR for the presence of *F. hepatica* infection in farms from September 2007 to February 2008 in Thessaly was high in the western area and in the eastern coast (Fig. 2B). A model-based predicted RR for the presence of *F. hepatica* in farms for 2007 in Greece was also constructed. The areas where the disease was most likely to be found were in the western region, the eastern coast, and the north-eastern region of the country (Fig. 3).

The developed risk model is a simple model based only on environmental datasets easily accessible to managers and available across broad geographical regions. Such information is of particular use to practitioners looking to extrapolate the results of prevalence risk studies. Since the chosen model was based on a single environmental variable (NDVI) which could be mapped across the entire Mediterranean basin, it was possible to extrapolate the model results to the whole Mediterranean region (Fig. 4).

DISCUSSION

Fasciolosis is a global problem for farmers and veterinarians because of its effect on meat, milk and wool production. More recently, it has become apparent that anthelmintic treatment is not always

Table 3. Analysis of herd and farmer characteristics association with occurrence of *Fasciola hepatica* infection in sheep and goat farms by coproantigen and high seropositivity

(Results of the two components of the model are presented as mean, odd ratios (OR) and 95% confidence intervals (CI).)

		Coproantigen (Component 1)			High seropositivity (Component 2)		
	N[1]	*n*[2] (%)	Mean (95% CI)	OR (95% CI)	*n* (%)	Mean (95% CI)	OR (95% CI)
Herd characteristics							
Species of farm animal							
Sheep	40	8 (20)	NS[3]	1	22 (55)	3·59 (0·64, 7·51)	1
Goat	34	4 (12)	− 9·54 (− 18·02, −0·17)	0·14 (7×10^{-6}, 0·75)	7 (21)	NS	0·76 (0·30, 1·00)
Breed of farm animal							
Sheep-Mountain type	11	5 (45)	19·34 (5·23, 26·61)	1	9 (82)	NS	—
Sheep-Chios	6	1 (17)	− 20·05 (− 50·83, −0·18)	2×10^{-6} (10^{-15}, 2×10^{-5})	1 (17)	NS	—
Sheep-other breeds	15	3 (20)	NS	—	8 (53)	NS	—
Goat-*Capra prisca*	29	4 (14)	NS	—	6 (21)	NS	—
Goat-Skopelos	6	0 (0)	NS	—	1 (17)	NS	—
Type of farming							
Organic	34	3 (9)	–11·55 (–23·05, –0·38)	1	14 (41)	3·47 (1·01, 6·81)	1
Conventional	40	9 (23)	NS	979 (0·35, 855 900)	15 (38)	NS	0·69 (0·35, 0·98)
Farm size (animals)							
Small (0–150)	20	5 (25)	NS	1	11 (55)	9·31 (4·52, 14·15)	1
Medium (151–300)	16	3 (19)	NS	—	6 (38)	NS	0·34 (0·007, 0·64)
Large (301–1800)	38	5 (13)	–7·68 (–13·99, –0·54)	112 (0·03, 4203)	12 (32)	NS	—
Farm milk production	NA[4]	NA	–0·05 (–0·10, –0·02)	1·05 (1·04, 1·08)	NA	NS	—
Farmer characteristics							
Age of farmer							
⩽ 45 years old	32	8 (25)	5·77 (2·54, 9·42)	1	14 (44)	3·38 (0·06, 6·51)	1
> 45 years old	42	5 (12)	NS	0·19 (0·001, 0·69)	15 (36)	NS	0·93 (0·81, 0·99)
Education level							
Compulsory school	51	6 (12)	NS	—	22 (43)	NS	1
Higher	23	6 (26)	NS	—	7 (30)	–5·69 (–13·13, –0·78)	0·009 (0·003, 0·031)

[1] *N*, Total number of farms; [2] *n*, Number of infected farms.

[3] NS, Not significant.

[4] NA, Not applicable (Continuous value).

Table 4. Analysis of farm management practices association with occurrence of *Fasciola hepatica* infection in sheep and goat farms by coproantigen and high seropositivity

(Results of the two components of the model are presented as mean, odd ratios (OR) and 95% confidence intervals (CI).)

		Coproantigen (Component 1)			High seropositivity (Component 2)		
Farm management practices	N^1	n^2 (%)	Mean (95% CI)	OR (95% CI)	n (%)	Mean (95% CI)	OR (95% CI)
Frequency of manure removal							
Once a year	35	6 (17)	−6·15 (−11·70, −0·25)	1	14 (40)	−3·44 (−5·46, −1·21)	1
Twice a year	23	4 (17)	−18·61 (−30·61, −9·14)	2×10^{-4} (10^{-7}, 0·001)	9 (39)	−9·22 (−12·59, −4·12)	0·04 (0·002, 0·24)
Never	7	1 (14)	NS[3]	—	3 (43)	NS	—
Frequency of veterinary monitoring of farm							
Never	9	1 (11)	NS	—	3 (33)	NS	1
Randomly	60	6 (10)	NS	—	22 (37)	NS	—
Every month	6	1 (17)	NS	—	0 (0)	−32·04 (−74·15, −7·77)	9×10^{-6} (10^{-33}, 2×10^{-4})
Farm equipment							
Poor	56	11 (20)	14·70 (6·60, 19·57)	1	22 (39)	NS	1
Sufficient	18	2 (11)	NS	0·58 (5×10^{-5}, 0·99)	7 (39)	3·01 (0·04, 6·42)	8·58 (1·46, 36·91)
Anthelmintic treatment							
No	17	3 (18)	NS	1	5 (29)	NS	—
Yes	57	9 (16)	−3·71 (−7·49, −0·09)	0·28 (0·08, 0·77)	24 (42)	NS	—
Frequency of anthelmintic administration							
Once	33	7 (21)	13·86 (5·68, 19·24)	1	14 (42)	NS	—
Twice per year	24	2 (8)	NS	0·83 (0·05, 0·99)	10 (42)	NS	—
Animal age during anthelmintic administration							
<1 year	3	0 (0)	NS	—	0 (0)	NS	1
>1 year	12	3 (25)	NS	—	5 (42)	NS	—
In any age	42	6 (14)	NS	—	19 (45)	3·78 (0·90, 7·88)	4×10^{7} (0·89, 4×10^{28})
Herd contact with other herds							
No	12	1 (8)	−34·38 (−51·46, −4·12)	1	4 (33)	NS	—
Yes	62	11 (18)	NS	0·01 (6×10^{-8}, 30510)	25 (40)	NS	—

[1] N, Total number of farms; [2] n, Number of infected farms.
[3] NS, Not significant.

Table 5. Analysis of pasture management association with occurrence of *Fasciola hepatica* infection in sheep and goat farms by coproantigen and high seropositivity

(Results of the two components of the model are presented as mean, odd ratios (OR) and 95% confidence intervals (CI).)

		Coproantigen (Component 1)			High seropositivity (Component 2)		
Pasture management	*N*[1]	*n*[2] (%)	Mean (95% CI)	OR (95% CI)	*n* (%)	Mean (95% CI)	OR (95% CI)
Permanent pasture							
Yes	49	9 (18)	NS[3]	1	19 (39)	NS	—
No	21	2 (10)	−16·56 (−26·32, −0·25)	0·20 (0·005, 0·69)	10 (48)	NS	—
Duration of grazing in months per year							
12 months	51	8 (16)	NS	1	18 (35)	NS	1
3–6 months	20	3 (15)	−6·66 (−11·84, −0·48)	0·27 (0·007, 1·12)	11 (55)	−4·01 (−6·18, −1·98)	0·41 (0·05, 1·43)
Never	3	1 (33)	NS	—	0 (0)	NS	—
Type of pasture							
Private	10	1 (10)	19·12 (4·22, 33·87)	1	3 (30)	NS	—
Public	42	5 (12)	NS	0·07 (10^{-5}, 0·57)	16 (28)	NS	—
Both	20	5 (25)	NS	—	10 (50)	NS	—
Pasture moisture							
Dry	45	4 (9)	NS	—	12 (27)	NS	1
Wet	25	7 (28)	NS	—	17 (68)	5·74 (2·58, 9·35)	38·03 (5·19, 131·9)
Water supply on pasture							
Tap water	17	2 (12)	NS	—	3 (18)	−11·76 (−18·72, −3·16)	1
Stream/river	22	1 (5)	NS	—	11 (50)	−6·24 (−11·57, −1·40)	10 (1·50, 1091)
Spring	15	1 (7)	NS	—	7 (47)	NS	—
Artificial lake	17	7 (41)	NS	—	8 (47)	NS	—
Water supply on farm							
Tap water	61	11 (18)	NS	—	20 (33)	NS	—
Stream/river	3	1 (33)	NS	—	2 (67)	NS	—
Spring	10	0 (0)	NS	—	7 (70)	NS	—

[1] *N*, Total number of farms; [2] *n*: Number of infected farms.
[3] NS, Not significant.

effective due to the development of drug resistance (Mitchell *et al*. 1998; Moll *et al*. 2000; Coles, 2005). Identifying the risk factors for *F. hepatica* infection may lead to the development of appropriate control measures for reducing the incidence of infection as well as the need for treatment in order to increase the efficiency of milk and meat production of animals.

Previous studies carried out elsewhere indicated a wide range of seroprevalence for ovine fasciolosis (Moghaddam *et al*. 2004; Mekroud *et al*. 2004). These differences are probably due to agro-ecological and climatic differences between the localities, although differences in the management systems may also have resulted in such variation (Abunna *et al*. 2010). In Italy, a neighbouring country of Greece, the prevalence of infection on sheep farms was estimated at 4·1% by faecal egg count (Cringoli *et al*. 2002), while in our study it was measured at 16·2% by coproantigen.

The statistical analysis showed that the correlation between the probability of a farm being infected as determined either by coproantigen or high seropositivity is very high (0·97). Ignoring this correlation and independently calculating the probabilities of a farm being infected as determined by coproantigen and high seropositivity would induce bias. It should be noted that coproantigen indicates carrier infection, while serology would indicate any past or recent exposure.

In regard to host species, the prevalence was significantly lower in goat than in sheep farms. This finding may be linked to the grazing habits of the two animal species: goats graze on leaves and branches on bushes and trees but sheep graze on plants on the ground where metacercaria are mostly found. So, the possibility of infection with metacercaria is higher in sheep than in goats. This observation is in agreement with other studies in Morocco (Alasaad *et al*. 2008) and in Argentina (Issia *et al*. 2009). In addition, differences in the prevalence of *F. hepatica* between breeds of sheep and goats noted in the present study were also observed by other investigators (Boyce *et al*.

Table 6. Analysis of environmental factors association with occurrence of *Fasciola hepatica* infection in sheep and goat farms by coproantigen and high seropositivity

(Results of the two components of the model are presented as mean, odd ratios (OR) and 95% confidence intervals (CI).)

Environmental factors	*N*[1]	Coproantigen (Component 1)			High seropositivity (Component 2)		
		n[2] (%)	Mean (95% CI)	OR (95% CI)	*n* (%)	Mean (95% CI)	OR (95% CI)
Season of sampling							
Autumn	34	3 (9)	NS[3]	—	13 (38)	NS	—
Winter	40	11 (28)	NS	—	16 (40)	NS	—
Elevation of farm location	NA[4]	—	−0·04 (−0·06, −0·02)	1·03 (1·02, 1·04)	—	NS	—
LST[5]	NA	—	−0·02 (−0·04, −0·01)	1·02 (1·01, 1·03)	—	NS	—
R[6]	NA	—	0·01 (7×10^{-4}, 0·02)	0·99 (0·99, 1)	—	NS	—
NDVI[7]	NA	—	NS	—	—	5·04 (0·46, 11·49)	0·98 (0·96, 0·99)
Coordinates of farm location							
Longitude	NA	—	0·26 (0·02, 0·39)	0·9996 (0·9978, 0·9999)	—	−0·09 (−0·17, −0·04)	1·06 (1·01, 1·10)
Latitude	NA	—	0·07 (0·01, 0·14)	0·996 (0·993, 0·999)	—	NS	—

[1] *N*, Total number of farms; [2] *n*, Number of infected farms.
[3] NS, Not significant.
[4] NA, Not applicable (Continuous value).
[5] LST, Average monthly land surface temperature (Kelvin) of farm location for 12 months before sampling.
[6] R, Monthly rainfall (mm) of farm location for 12 months before sampling.
[7] NDVI, Average NDVI of farm location for 12 months before sampling.

1987; Khallaayoune *et al.* 1991). The apparent influence of breed is perhaps closely associated with the husbandry system (Sanchez-Andrade *et al.* 2002). For example, sheep of the mountain-type breed graze in pastures while sheep of the Chios breed are mostly housed permanently.

Farms that use private and permanent pastures have a significantly higher risk of getting infected with *F. hepatica* compared to farms where animals graze on public pastures. Private pastures have a small area compared to public pastures and animals graze on them for a long period of time. So, there is a constant shedding of eggs on these pastures. In addition, private pastures are usually irrigated and irrigation has been found to be a significant risk factor for the presence of fasciolosis in cattle, as documented in a study by Durr *et al.* (2005).

Wet pastures with mud appeared to be a significant risk factor. This is expected since this kind of environment is appropriate for the propagation (survival) of snails. Local seasonal crowding of animals along the banks of water provides an important opportunity for transmission (Njau *et al.* 1989). The water supply of animals in the present study also appeared to be a significant factor for the presence of *F. hepatica*; when livestock drink tap water, farms had a lower risk of infection.

The variables introduced into the statistical model that concern the age and the educational level of farmers had also been investigated by Cringoli *et al.* (2002) but they were not found to be significant. In the current study, the age of farmers was recognized as a protective factor but the educational level as a risk factor. Perhaps, older farmers may have experience in local conditions and better stockmanship skills.

The results of the model suggest that the risk factors were not determined only by herd characteristics, farmer status, farm and pasture management but also by environmental factors. *F. hepatica* in Greece occupies a climate range, which is mostly warm and dry. Therefore, parasite development and snail reproduction are less constrained by low temperature; but they are constrained by lack of moisture resulting in breaks in the life-cycle of the parasite (Boray, 1969). The western part of Thessaly consists of mountains with higher moisture and NDVI values than in the eastern part. These climatic conditions are appropriate for the development of the intermediate host (Urquhart *et al.* 1987) and furthermore, moisture is considered to be an important factor that determines the survival and availability of snails.

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

Table 7. Significant clusters of *Fasciola hepatica* infections in Thessaly, Greece

Cluster	Locality	Number of cases	Expected cases	Relative risk	*P*-value
Most likely	Vrynena	11	2·15	5·70	0·0004
Secondary	Labero, Ag. Georgios, Mitropoli, Rousso, Mouzaki	19	7·41	2·99	0·0045
Secondary	Vlachogianni	9	2·68	3·62	0·0725

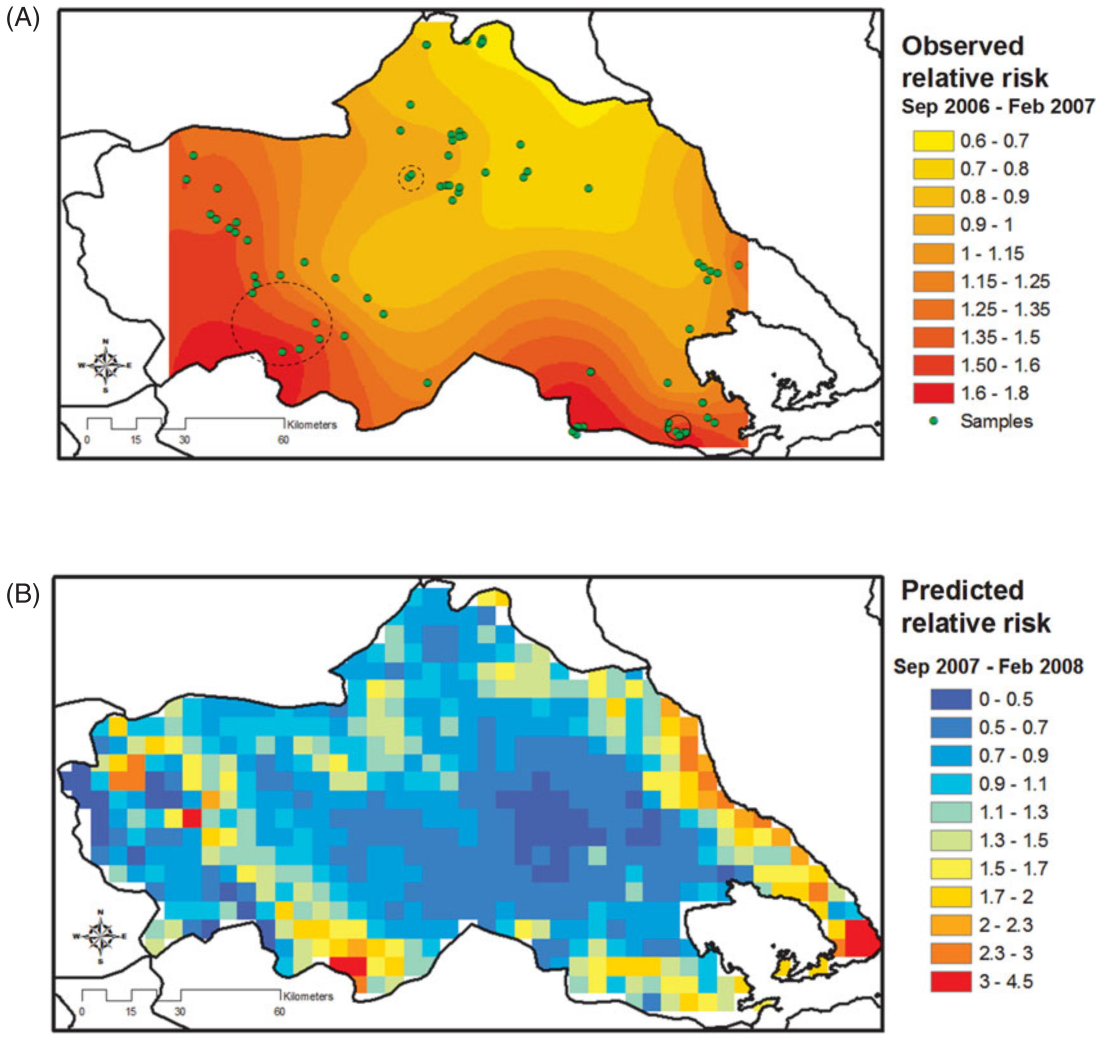

Fig. 2. (A) Map of observed relative risk of *Fasciola* infection in Thessaly during the sampling period (September 2006 to February 2007), representing the effect of NDVI of a farm adjusting for the other significant factors. The kriging spatial interpolation method (Oliver, 1990) was used to estimate relative risk values 'in between' sampled values. Non-shaded areas are outside the extent of the sample sites' locations and were excluded from the spatial interpolation domain. The circles enclose the locations identified within each cluster of *Fasciola* infection (Continuous line: most likely cluster; Dashed line: secondary cluster). (B) Map of predicted relative risk of *Fasciola* infection in Thessaly for each NDVI value for the following years (September 2007 to February 2008), adjusting for the other significant factors.

significant environmental risk factor for *F. hepatica* infection in the component 2 of the model. This result is in agreement with the study of Durr *et al.* (2005) that took place in similar climatic conditions in Australia. Various studies have indicated the significance of NDVI data for the construction of predictive models in Africa (Malone *et al.* 1998; Yilma and Malone, 1998), South America (Fuentes, 2006), and USA (Zukowski *et al.* 1993). These models generated 'health maps' that were used in routine disease control programmes (Malone *et al.* 1998). In the present study, the model indicated that the highest RR in Thessaly was in the south-eastern and western areas. This observation was confirmed by the cluster analysis which showed that 2 likely clusters of infection were present in the western and south-eastern areas. Moreover, the significance of NDVI in the model may be a reflection of the environmental

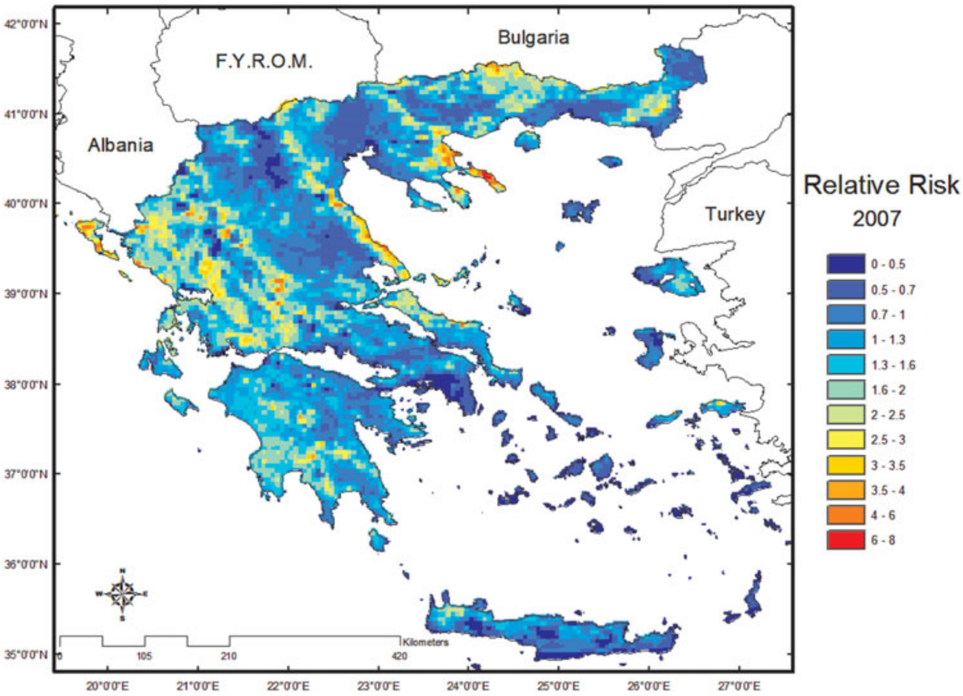

Fig. 3. Map of predicted relative risk of *Fasciola* infection in Greece during 2007. The model was constructed based on NDVI values detected in Greece, adjusting for the other significant factors.

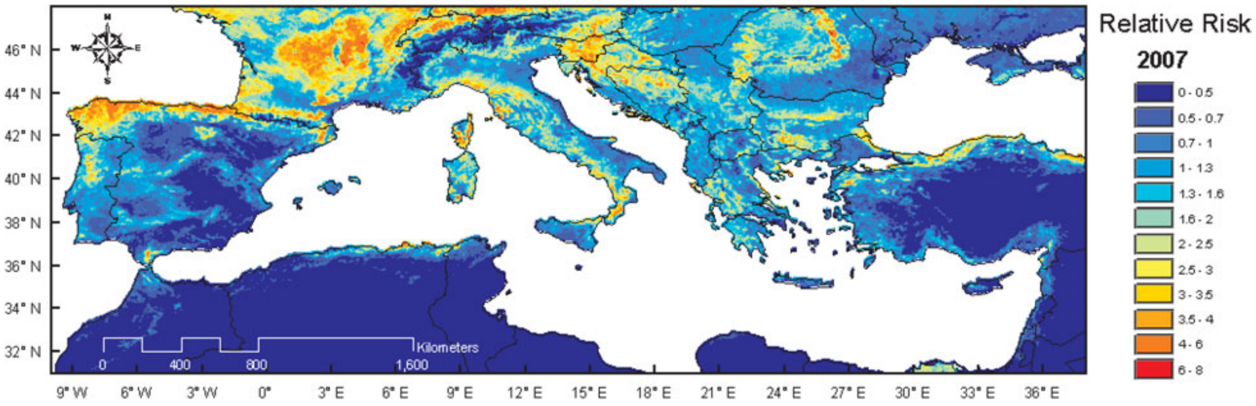

Fig. 4. Map of predicted relative risk of *Fasciola* infection in Mediterranean basin during 2007. The model was constructed based on NDVI value in the 0·25–0·81 range, adjusting for the other significant factors.

requirements of the snail vectors of the parasite. NDVI as a surrogate of climatic risk data can be included in a GIS as separate layers on long-term climate pattern, and maps of annual values (Yilma and Malone, 1998). NDVI values integrate a number of different environmental factors (land cover, temperature, rainfall, vapour pressure, etc.) into a single variable and thus simplifies the analysis (Hay *et al.* 1997). In South America, the predicted risk map based on NDVI has been shown to present a comprehensive GIS control program model that accurately fits real epidemiological and transmission situations of human fasciolosis (Fuentes *et al.* 2001).

The map of predicted RR in Thessaly showed that the most likely areas of *Fasciola* infection in sheep and goat farms were the eastern coast where the climate was influenced by the sea and the NDVI values were high. The observed risk areas and the predicted risk areas were almost identical in the west part of Thessaly. This is a semi-mountainous area with frequent rainfall, which is appropriate for the survival of the intermediate host.

A technique gaining popularity in spatial epidemiology is to develop regression models on a given study area and then utilize the estimated relationship to enable extrapolation over a much wider area (Cringoli *et al.* 2004; Fuentes, 2006). On the other hand, extrapolation results should always be used with caution, as in the current study, since all the examined factors in the region of Thessaly were considered to be similar in the entire area of Greece. In the present study this technique was applied to develop a predictive RR map concerning not only the studied region but Greece as a whole, as the NDVI values in Thessaly are representative for the whole country. The model showed that the predicted RR of *Fasciola* infection in Greece was high in the western area, where the largest sheep (43%) and goat (32%) populations are located (HSA, 2006). The western part of Greece is a mountainous area characterized by higher rainfall and NDVI values than the surrounding lowlands. The economy of this region is largely based on animal farming and therefore farmers should be aware of the importance of fasciolosis for small ruminants and other livestock such as cattle, and additionally for human health (Rojas *et al*. 2010).

It should be noted that the derived risk map for the whole Mediterranean region is only indicative and is used to present the outcome of the application of our model to other areas in the Mediterranean basin. Given that the data used for estimating the model stem from a small area within Greece, one needs to be particularly cautious in extrapolating the predicted risk to other regions, as has been done here. Even though the Mediterranean region is affected by the 'Mediterranean climate', significant differences in temperature and rain may exist between north and south as well as between inland and coastal areas. In addition, we used a number of factors that were area-specific. This suggests that a different model could be obtained if similar studies were conducted elsewhere in the Mediterranean. The accuracy of our extrapolation could be tested via an external validation test but this was out of the scope of the present paper.

In conclusion, this study demonstrated that Thessaly should be regarded as an endemic region for *Fasciola* infection and it represents the first prediction model of *Fasciola* infection in small ruminants in the Mediterranean basin. The identified risk factors and the prediction model can be useful to formulate appropriate control strategies for fluke prevention in sheep and goats in Greece and other Mediterranean countries.

ACKNOWLEDGEMENTS

Positive sera from sheep experimentally infected with *F. hepatica* and negative sera from clinically healthy sheep were kindly supplied by Dr D. J. Williams, Veterinary Parasitology, Department of Veterinary Pathology, School of Veterinary Science, University of Liverpool UK. Positive sera from goats experimentally infected with *F. hepatica* and negative sera from clinically healthy goats were kindly supplied by Dr J. Perez and A. Martinez-Moreno, Department of Veterinary Clinical Science, Faculty of Veterinary Science, University of Cordoba, Spain.

FINANCIAL SUPPORT

This work was supported by the European Union-funded DELIVER Project (Contract No.: FOOD-CT-2004-023025).

REFERENCES

Abunna, F., Asfaw, L., Megersa, B. and Regassa, A. (2010). Bovine fasciolosis: coprological, abattoir survey and its economic impact due to liver condemnation at Soddo municipal abattoir, Southern Ethiopia. *Tropical Animal Health and Production* **42**, 289–292.

Alasaad, S., Granados, J. E., Cano-Manual, F. J., Meana, A., Zhu, X. Q. and Perez, J. M. (2008). Epidemiology of fasciolosis affecting Iberian ibex (*Capra pyrenaica*) in southern Spain. *Parasitology Research* **102**, 751–755.

Antoniou, M., Lionis, C. and Tselentis, Y. (1997). A focus of *Fasciola hepatica* in Crete without human cases. *European Journal of Epidemiology* **13**, 99–101.

Beck, L. R., Lobitz, B. M. and Wood, B. L. (2000). Remote sensing and human health: new sensors and new opportunities. *Emerging Infectious Diseases* **6**, 217–227.

Bennema, S. C., Ducheyne, E., Vercruysse, J., Claerebout, E., Hendrickx, G. and Charlier, J. (2011). Relative importance of management, meteorological and environmental factors in the spatial distribution of *Fasciola hepatica* in dairy cattle in a temperate climatic zone. *International Journal for Parasitology* (in the Press) (doi:10.1016/j.ijpara.2010.09.003).

Bennema, S. C., Vercruysse, J., Claerebout, E., Schnieder, T., Strube, C., Ducheyne, E., Hendrickx, G. and Charlier, J. (2009). The use of bulk – tank milk ELISA to assess the spatial distribution of *Fasciola hepatica, Ostertagia ostertagi* and *Dictyocaulus viviparous* in dairy cattle in Flanders (Belgium). *Veterinary Parasitology* **165**, 51–57.

Boray, J. C. (1969). Experimental fasciolosis in Australia. *Advances in Parasitology* **7**, 95–210.

Boyce, W. M., Courtney, C. H. and Loggins, P. E. (1987). Resistance to experimental infections with *Fasciola hepatica* in exotic and domestic breeds of sheep. *International Journal for Parasitology* **17**, 1233–1237.

Coles, G. C. (2005). Anthemintic resistance – looking to the future: a UK perspective. *Research in Veterinary Science* **78**, 99–108.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G. and Malone, J. B. (2002). A cross-sectional coprological survey of liver flukes in cattle and sheep from an area of southern Italian Apennines. *Veterinary Parasitology* **108**, 137–143.

Cringoli, G., Taddei, R., Rinaldi, L., Veneziano, V., Musella, V., Carcone, C., Sibilio, G. and Malone, J. B. (2004). Use of remote sensing and geographical information systems to identify environmental features that influence the distribution of paramphistomosis in sheep from the southern Italian Apennines. *Veterinary Parasitology* **122**, 15–26.

Durr, P. A., Tait, N. and Lawson, A. B. (2005). Bayesian hierarchical modelling to enhance the epidemiological value of abattoir surveys for bovine fasciolosis. *Preventive Veterinary Medicine* **71**, 157–172.

Dwass, M. (1957). Modified randomization tests for nonparametric hypotheses. *Annals of Mathematical Statistics* **28**, 181–187.

Feidas, H. (2010). Validation of satellite rainfall products over Greece. *Theoretical and Applied Climatology* **99**, 193–216.

Fuentes, M. V. (2006). Remote sensing and climate data as a key for understanding fasciolosis transmission in the Andes: a review and update of an ongoing interdisciplinary project. *Geospatial Health* **1**, 59–70.

Fuentes, M. V., Malone, J. B. and Mas-Coma, S. (2001). Validation of a mapping and prediction model for human fasciolosis transmission in Andean very high altitude endemic areas using remote sensing data. *Acta Tropica* **79**, 87–95.

Hay, S. I., Pacher, M. J. and Rogers, D. J. (1997). The impact of remote sensing on the study and control of invertebrate intermediate hosts and vectors for disease. *International Journal of Remote Sensing* **15**, 2899–2930.

Hillyer, G. V. (2005). *Fasciola* antigens as vaccines against *fascioliasis* and *schistosomiasis*. *Journal of Helminthology* **79**, 241–247.

HSA (2006). Hellenic Statistical Authority (http://www.statistics.gr)

Huffman, G. J., Adler, R. F., Bolvin, D. T., Gu, G., Nelkin, E. J., Bowman, K. P., Hong, Y., Stocker, E. F. and Wolff, D. B. (2007). The TRMM multisatellite precipitation analysis: quasi-global, multi-year, combined-sensor precipitation estimates at fine scale. *Journal of Hydrometeorology* **8**, 38–55.

Issia, L., Pietrokovsky, S., Sousa-Figueiredo, J., Sttothard, R. and Wisnivesky-Colli, C. (2009). *Fasciola hepatica* infections in liverstock flock, guanacos and coypus in two wildlife reserves in Argentina. *Veterinary Parasitology* **165**, 341–344.

Khallaayoune, K. H., Stromberg, B. E., Dakkak, A. and Malone, J. B. (1991). Seasonal dynamics of *Fasciola hepatica* burdens in grazing Timahdit sheep in Marocco. *International Journal for Parasitology* **21**, 307–314.

Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics – Theory and Methods* **26**, 1481–1496.

Kulldorff, M. and Nagarwalla, N. (1995). Spatial disease clusters: detection and inference. *Statistics in Medicine* **15**, 707–715.

Loukas, A., Vasiliades, L. and Tzarbiras, J. (2007). Evaluation of climate change on drought impulses in Thessaly, Greece. *European Water* **17/18**, 17–28.

Lunn, D. J., Thomas, A., Best, N. and Spiegelhalter, D. (2000). WinBUGS – a Bayesian modelling framework: concepts, structure, and extensibility. *Statistics and Computing* **10**, 325–337.

Malone, J. B., Gommes, R., Hansen, J., Yilma, J. M., Slingenboerg, J., Snijders, F., Nachtergaele, F. and Ataman, E. (1998). A geographical information system on the potential distribution and abundance of *Fasciola hepatica* and *F. gigantica* in east Africa on food and agriculture organization databases. *Veterinary Parasitology* **78**, 87–101.

Malone, J. B., Yilma, J. M., McCarroll, J. C., Erko, B., Mukaratirwa, S. and Zhou, X. (2001). Satellite climatology and the environmental risk of *Schistosoma mansoni* in Ethiopia and east Africa. *Acta Tropica* **79**, 59–72.

Mas-Coma, S. (2004). Human fasciolisis. In *Waterborne Zoonoses: Identification, Causes and Control* (ed. Cotruvo, J. A., Dufour, A., Ress, G., Bartram, J., Carr, R., Cliver, D. O., Craun, G. F., Fayer, R. and Gannon, V. P. J.), pp. 209–212. World Health Organization (WHO), IWA Publishing, London, UK.

Mas-Coma, S. and Bargues, M. D. (1997). Human liver flukes: a review. *Research and Reviews in Parasitology* **57**, 145–218.

McCann, C. M., Baylis, M. and Williams, D. J. L. (2010). The development of linear regression models using environmental variables to explain the spatial distribution of *Fasciola hepatica* infection in dairy herds in England and Wales. *International Journal for Parasitology* **40**, 1021–1028.

Mekroud, A., Benakhla, A., Vignoles, P. and Rondelaud, D. (2004). Preliminary studies on the prevalence of natural fasciolosis in cattle, sheep and the host snail (*Galba truncatula*) in north-eastern Algeria. *Parasitology Research* **92**, 502–505.

Mitchell, G. B. B., Maris, L. and Bonniwell, M. A. (1998). Triclabendazole-resistant liver fluke in Scottish sheep. *Veterinary Record* **143**, 399.

Moghaddam, A. S., Massoud, J., Mahmoodi, M., Mahvi, A. H., Periago, M. V., Artigas, P., Fuentes, M. V., Bargues, M. D. and Mas-Coma, S. (2004). Human and animal fascioliasis in Mazandaran province, northern Iran. *Parasitology Research* **94**, 61–69.

Moll, L., Gaasenbeek, C. P. H., Vellema, P. and Borgsteede, F. H. M. (2000). Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in the Netherlands. *Veterinary Parasitology* **91**, 153–158.

Neteler, M. (2004). MODIS time series remote sensing for epidemiological modelling. International Symposium on Geoinformatics for Spatial Infrastructure Development in Earth and Allied Sciences 2004.

Njau, B. C., Kassali, O. B., Scholtens, R. G. and Akalework, N. (1989). The ingluence of watering practices on the transmission of *Fasciola* among sheep in the Ethiopia highlands. *Veterinary Research Communications* **13**, 67–74.

Oliver, M. A. (1990). Kriging: a method of interpolation for Geographical Information Systems. *International Journal Of Geographical Information Systems* **4**, 313–332.

Roberts, J. A. and Suhardono (1996). Approaches to the control of fasciolosis in ruminants. *International Journal for Parasitology* **26**, 971–981.

Rojas, L., Vazquez, A., Domenech, I. and Robertson, L. J. (2010). Fascioliasis: can Cuba conquer this emerging parasitosis? *Trends in Parasitology* **26**, 26–34.

Salimi-Bejestani, M. R., McGarry, J. W., Felstead, S., Ortiz, P., Akca, A. and Williams, D. J. (2005). Development of an antibody-detection ELISA for *Fasciola hepatica* and its evaluation against a commercially available test. *Research in Veterinary Science* **78**, 177–81.

Sanchez-Andrade, R., Paz-Silva, A., Suarez, J. L., Panadero, R., Pedreira, J., Lopez, C., Diez-Banos, P. and Morrondo, P. (2002). Influence of age and breed on natural bovine fasciolosis in an endemic area (Galicia, NW Spain). *Veterinary Research Communications* **26**, 361–70.

Spithill, T. W. and Dalton, L. P. (1998). Progress in development of liver fluke vaccines. *Parasitology Today* **14**, 224–228.

Theodoropoulos, G., Theodoropoulou, E., Petrakos, G., Kantzoura, V. and Kostopoulos, J. (2002). Abattoir condemnation due to parasitic infections and its economic implications in the region of Trikala, Greece. *Journal of Veterinary Medicine Series B* **49**, 281–284.

Tum, S., Puotinen, M. L. and Coppeman, D. B. (2004). A geographic information systems model for mapping risk of fascioliosis in cattle and buffaloes in Cambodia. *Veterinary Parasitology* **122**, 141–149.

Turnbull, B. W., Iwano, E. J., Burnett, W. S., Howe, H. L. and Clark, L. C. (1990). Monitoring for clusters of disease: application to leukemia incidence in upstate New York. *American Journal of Epidemiology* **132** (Suppl.) S136–S143.

Urquhart, G. M., Armour, J., Duncan, A. M. and Jennings, F. W. (1987). Veterinary Parasitology, 2nd ed. Longman Scientific & Technical, England, 104 pp.

Wan, Z. and Dozier, J. (1996). A generalized split-window algorithm for retrieving land-surface temperature from space. *IEEE Transactions on Geoscience and Remote Sensing* **34**, 892–905.

Yilma, J. M. and Malone, J. B. (1998). A geographical information system forecast model for strategic control of fasciolosis in Ethiopia. *Veterinary Parasitology* **78**, 103–127.

Zukowski, S. H., Wilkerson, G. W. and Malone, J. B. (1993). Fasciolosis in cattle in Lousiana. II. Development of a system to use soil maps in geographic information system to estimate disease risk on Louisiana coastal marsh rangeland. *Veterinary Parasitology* **47**, 51–65.