Bovine paramphistomosis in Galicia (Spain): prevalence, intensity, aetiology and geospatial distribution of the infection.

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Abstract

This study explored various aspects of the epidemiology of paramphistomosis in Galicia, the main cattle producing region in Spain. A total of 589 cows from different farms were selected in the slaughterhouse for examination of the forestomachs for the presence of Paramphistomidae flukes. Paramphistomes were found in 111/589 cows (18.8%; 95% CI: 15.7%-21.9%), with higher prevalences of infection in beef than in dairy cows (29.2% vs 13.9%). Although the number of flukes per animal was generally low (median=266), some cows harboured large burdens (up to 11895), which may have harmful effects on their productivity. Cows with higher parasite burdens also excreted greater numbers of eggs in faeces, so that heavily parasitized cows play an important role in the transmission of paramphistomosis. This role is especially relevant in Galicia, where the roe deer, which is the only wild ruminant in the study area, was found not to be a reservoir for the infection. The use of morpho-anatomical and molecular techniques provided reliable confirmation that *Calicophoron daubneyi* is the only species of the family Paramphistomidae that parasitizes cattle in Galicia. The environmental data from the farms were used in Bayesian geostatistical models to predict the probability of infection by *C.daubneyi* throughout the region. The results

revealed the role of environmental risk factors in explaining the geographical heterogeneity in the probability of infection in beef and dairy cattle. These explanatory factors were used to construct predictive maps showing the areas with the highest risk of infection and the uncertainty associated with the predictions.

Keywords

Paramphistomosis, *Calicophoron daubneyi*, cattle, roe deer, epidemiology, Bayesian geostatistical model

Introduction

Paramphistomosis is a parasitic infection caused by digenetic trematodes belonging to the family Paramphistomidae Fischoeder, 1901, which includes many genera and species (e.g. *Paramphistomum* spp., *Calicophoro*n spp., *Cotylophoron* spp., *Gigantocotyle* spp., etc.) that inhabit the gastrointestinal tract of wild and domestic ruminants throughout the world [1]. Paramphistomes have a heteroxenous life cycle that involves freshwater snails (intermediate hosts). The parasite larvae develop until reaching a stage (cercaria) that emerges from the mollusc and typically encysts on vegetation, hard surfaces or water, and then develops into a stage (metacercaria) that is ingested by grazing ruminants (final host). In the ruminant host, juvenile parasites first locate in the small intestine and feed on the intestinal mucosa. As they grow, the parasites migrate upwards to the reticulum and rumen where they spend the remainder of their adult lives, shedding eggs that contaminate snail habitats.

As regards the pathology caused by the paramphistomes in domestic ruminants, most authors consider that immature worms migrating in the small intestine can provoke severe damage, including death, whereas the adult flukes established in rumen and reticulum are not considered very harmful [2, 3, 4]. Nevertheless, ruminal lesions due to heavy infections by adult worms have been recorded [5, 4]. Digestion and absorption may be affected, resulting in diarrhoea, anorexia, anaemia and weakness [6, 7, 8]. The

84 effect of the infection on animal production remains controversial [9, 10], partly
85 because detrimental effects of paramphistomes can be masked by concurrent
86 infections with other helminths (e.g. *Fasciola hepatica* and gastrointestinal nematodes)
87 that usually infect grazing ruminants. Moreover, as with other parasitic infections, the
88 pathology of paramphistosis also depends on the immune status of infected animals,
89 the size of parasite burdens and the species of parasite involved in the infections [4,
90 11].
91 Paramphistomosis is highly prevalent in tropical and subtropical countries [2, 12, 13, 8]
92 where it causes high morbidity, mainly in animals raised by use of traditional husbandry
93 systems, in which periods of nutritional stress occur. In Europe, the presence of
94 paramphistomes in cattle had been considered virtually harmless [14]. However,
95 Dorchies et al. [3] have described some cases of serious illness caused by
96 paramphistomes. Furthermore, epidemiological studies conducted in central France
97 [15] have shown that the prevalence of infection with *Calicophoron daubneyi* [16, 17] in
98 cattle increased significantly, from 5.2% to 44.7%, between 1990 and 1999.
99 Paramhistomosis has also been detected on farms in UK and Wales [18] and Ireland
100 [19], indicating that this pathology should be considered in the differential diagnosis of
101 other enteric processes in cattle. In Spain, infections by *Paramphistomum cervi* have
102 been reported in cows and deer [20, 21]. Díaz et al. [22, 23] detected, by coprological
103 techniques, infections by paramphistomes in 26%-36% cattle farms in Galicia, which
104 appears to indicate a high prevalence of bovine paramphistomosis in the region.
105 However, documentation of the presence of infection and/or the raw data on its
106 prevalence, without any assessment of the spatial variability existing in such a wide
107 region, is of limited interest and does not indicate the real risk of infection in areas with
108 different climatic and environmental conditions, which may affect the life cycle of the
109 parasite. Moreover, other circumstances such as the existence of wild reservoirs for
110 paramphistomes have not been evaluated.

111 All of the above led us to carry out a comprehensive epidemiological study on cattle
112 paramphistomosis in Galicia, which included the following steps: 1) at bovine host
113 level, we determined the parasite burdens in rumens and reticula as well as the
114 distribution of the parasites within these organs. We also identified, by
115 morphoanatomical and molecular analysis, the species of Paramphistomidae worms
116 found at necropsy, and we determined the association between the amount of flukes in
117 the rumen and reticulum and the numbers of parasite eggs in faecal samples; 2) at the
118 host level, i.e. in roe deer (*Capreolus capreolus*), which is the only wild ruminant found
119 in the vicinity of cattle farms, we performed necropsies to detect the presence of
120 paramphistomes; and 3) at geospatial level, we investigated the distribution of the
121 cattle paramphistomosis, exploring the linkages between the spatial patterns and some
122 climatic and environmental factors that are thought to affect different phases of the
123 parasite life cycle (e.g. survival and distribution of the intermediate host and its
124 probability of infection, the development of the intra-mollusc larval stages and the
125 infection risk of cattle, among other factors). Moreover, we constructed predictive maps
126 to help decision-makers spatially target the monitoring and control of paramphistomosis
127 in dairy and beef cattle. All statistical analyses were performed in a Bayesian
128 framework to incorporate uncertainty about model parameters.
129
130 ## Materials and methods
131 ### *Study area*
132 The present study was carried out in Galicia (NW Spain), which is located between
133 latitudes 41º 49' to 43º 47'N and longitudes 6º 42' to 9º 18'W, and surrounded by the
134 Cantabria Sea to the north and the Atlantic Ocean to the west. The climate in the
135 region is temperate maritime, with an annual average temperature of 11.6 ºC and
136 annual average rainfall of 1065 mm. The altitude ranges between 0 and 2129 m. The
137 soil mainly consists of metamorphic and igneous rocks, which in hydrological terms
138 represents a very heterogeneous and anisotropic environment.

Galicia covers a total surface area of 29575 km^2, administratively divided into 315 municipalities with very different cattle farming activity and stocking rate per surface unit. According to the 2008 livestock census, there were 339530 dairy cows (99% on farms in the northern half of the region), and 221917 beef cows (on farms spread over a larger area extending to the South-East of the region). Grasslands occupy approximately 60% of the useful agricultural land and cows usually graze throughout the year, mainly on beef cattle farms. The type of livestock husbandry and the climatic characteristics of the region favour grazing-linked transmission of helminthosis.

Sampling

A slaughterhouse that processes cattle from the whole region was visited fortnightly during 2008. At each visit, 20 adult cows (over 2 years old) were selected at random to determine the existence of infections by paramphistomes within the rumens and/or reticula, as well as the occurrence of trematode eggs in the faeces. The rumen, reticula and stool samples from a total of 589 cows, all from different farms (Fig. 1), were removed and transported to the laboratory promptly.

The forestomachs from 235 roe deer killed during the 2008 hunting season throughout Galicia were also collected and examined for the presence of flukes.

Parasitological techniques

Each forestomach was thoroughly examined for recovery and counting of flukes; moreover, in 75 out of the parasitized cows, the anatomic distribution of flukes within the rumen (atrium, rumenoreticular sulcus, ventral sac and dorsal sac) was also recorded.

Species identification was carried out by conventional microscopy and subsequent confirmation by molecular techniques. A sample of 50 paramphistomes (or all of those present, if parasite burdens was not as high as this) was collected at random from each parasitized cow and then split into 2 subsamples. The parasites in one of the

subsamples were preserved in 70% ethanol until being stained for microscopic examination, while specimens in the other subsample were frozen individually at -85 ºC until extraction of the DNA for molecular analysis by PCR. In those 75 cows in which the location of the parasites was recorded, samples were taken from each anatomical region following the pattern described. Species were identified as follows:

1) A total of 618 randomly selected alcohol-fixed specimens (from different cows and anatomical locations) were stained with borax carmine, mounted on permanent slides and examined by microscopy and microphotography in order to measure the length, width and area of the whole body, the oral and ventral suckers, the anterior and posterior testis and the ovary. The species were identified according to the criteria outlined by Dinnik, J.A. [16] and Eduardo, S.L. [17].

2) The species identity was further confirmed by molecular analysis of 82 individually frozen parasite specimens. Samples of DNA extracted from 82 specimens were analyzed as reported by Martínez-Ibeas et al. [25]. Briefly, the second internal transcribed spacer (ITS-2) region of ribosomal DNA from each of the individual flukes was amplified by PCR, with primers specific to the species, previously identified by morphological techniques (F: 5' TGCATACTGCTTTGAACATCG 3' and R: 5' GTTCAGCGGGTATTCACGTC 3'). The sequences of the amplification products were compared with those available in Genbank™ for species identification.

Faeces were analysed by a standard sedimentation technique. Faecal samples (10 g) were first subjected to 3 successive sedimentation processes, and the sediments were then examined by microscopy to count the paramphistomid eggs. The analytical sensitivity of this technique was 2 eggs per gram of faeces (epg).

Factors potentially associated with infections by paramphistomes

A total of 10 variables were tested for their potential association with bovine paramphistomosis. Data on cow-related variables, such as age, type of production (milk or beef) and livestock density (cows/km^2 in year 2008) in the municipalities where

source farms were located were provided by the Galician Animal Production Service. Data on environmental variables (land cover, elevation, slope, type of soil and soil permeability) for the geographical coordinates of originating farms were provided by the Galician Cartographic Office (SITGA). The land cover data supplied corresponded to the following 8 categories of the CORINE Land Cover map: 1: forest, 2: permanent crops, 3: arable land, 4: pastures, 5: wetland, 6: shrub, 7: mines and 8: urban zones. Data on elevation (m) and slope (%) were obtained from a 5-meter resolution Digital Elevation Model (DEM), which was created by the National Geographic Institute of Spain, within the National Project of Aerial Orthophotography. The source of lithological data for soil classification was the 1:50000 digital geological map constructed by the Geological and Mining Institute of Spain (MAGNA project). According to this map, there are 10 different soil types in Galicia: 1: limestone and dolomite, 2: acid igneous rock, 3: cenozoic deposits, 4: gneiss, 5: amphibolite, 6: slate and quartzite, 7: shale, 8: eclogite and granulite, 9: basalt and peridotite, and 10: serpentinite. The digital geolithological map was also used to create a permeability map from which soil permeability data were obtained and then grouped into three categories: 1: low permeability (≤ 0.5 cm/h), 2: medium permeability (0.5-12.5 cm/h) and 3: high permeability (> 12.5 cm/h).
The climatic variables included in the study were the annual mean temperature and total rainfall calculated from the data recorded at the 67 official weather stations in Galicia during the period 2004-2008 (www.meteogalicia.es). The average annual values were then used to make a kriging (1 km^2 grid), for which data for each farm coordinate were interpolated.

Statistical analysis

The prevalence of the Paramphistomidae flukes was first analyzed in terms of the type of cattle (beef or dairy) and age (less than 6 years; between 7 and 9 years; and more than 9 years) using a Generalized Linear Model (GLM) (specifically a logistic regression model, in which the response variable was a binary variable that represents

223 the presence or absence of the parasite in each cow sampled). We also used a GLM to
224 model the relationship between the positive coprology with the type of cattle and age. A
225 linear model was used to analyze the relationship between the total number of
226 paramphistomes observed in infected cows (in particular its logarithm) and the type of
227 cattle and age. A linear model was also used to analyze the relationship between the
228 number of eggs observed in infected cows (in particular, a Box-Cox transformation of
229 this variable) and the type of cattle and age. Finally, the relationship between the latter
230 two response variables was also analyzed by a Linear Model (with logarithmic
231 transformation of the parasite burden). As previously mentioned, the INLA methodology
232 was used to implement these five types of analysis within a Bayesian framework to
233 incorporate uncertainty about model parameters [24].
234 The spatial variation in the probability of infection in beef and dairy cattle was modelled
235 by a hierarchical Bayesian spatial approach (see 26), specifically a point-reference
236 spatial model [27]. These models are highly suitable for situations (as in the present
237 study) in which data are observed at continuous locations occurring within a defined
238 spatial domain (geo-referenced Bernoulli data). Note that these models can also be
239 considered as a spatial extension of logistic regression models because the modelling
240 process describes the variability in the response variable as a function of the
241 explanatory variables, with the addition of a stochastic spatial effect, which models the
242 residual spatial autocorrelation [28].
243 More specifically, the response variable is a binary variable that represents the
244 presence or absence of the parasite in each cow sampled: Z_i is 1 if cow i is infected
245 and 0 if not. Consequently, the conditional distribution of the data is $Z_i \sim Ber(\pi_i)$,
246 assuming that observations are conditionally independent given π_i, which is the
247 probability of occurrence at location i (I = 1,…,n).
248 At the first stage of the hierarchical model, we modelled the occurrence of parasite as a
249 GLM by using the usual (for binary data) logit link function, but incorporating a spatial
250 effect. That is,

$$logit\ (\pi_i) = X_i\beta + W_i$$

where β represents the vector of the regression coefficients, X is the matrix of covariates, W represents spatial random effects and the logit transformation is defined as $logit(\pi_i) = \log\left(\frac{\pi_i}{1-\pi_i}\right)$.

The second stage of the hierarchical model is used to incorporate the uncertainty about the parameters (before taking into account the observations) used in the first level. Following Bayesian reasoning, the parameters are treated as random variables, and prior knowledge is incorporated via prior distributions. In particular, for the parameters involved in the fixed effects, we use Gaussian distributions β~$N(0,0.01)$, parameterized by their precision (inverse of the variance).

In the third, and final, level of hierarchy, prior knowledge about the hyperparameters is expressed. The hyperparameters are all derived from the spatial effect. More precisely, we assume that W follows a prior Gaussian distribution with zero mean and covariance matrix depending of hyperparameters κ and τ, which determine the range of the spatial effect and the total variance, respectively.

As usual in this context, the resulting hierarchical Bayesian model has no closed expression for the posterior distribution of all the parameters, and so numerical approximations are needed. Here, we use the integrated nested Laplace approximation (INLA) methodology [24] and software (http:\\www.r-inla.org) as an alternative to the Markov chain Monte Carlo (MCMC) methods. The main reason for this choice is the speed of calculation and the possibility of comparing many different models [24].

Inference and prediction in unsampled locations were performed simultaneously by INLA. For this, we used the Stochastic Partial Differential Equation module [29], which allows us to fit the particular case of continuously indexed Gaussian Fields by INLA, as is the case with our spatial component. Once the inference is carried out, the next step is to predict the occurrence probability for paramphistomosis in the rest of the area of interest (Galicia in our case), especially in non-observed locations. Following the

277 Bayesian reasoning, the occurrence of the species at new locations are considered as
278 random variables, so that it is possible to obtain a set of probable values, together with
279 the probabilities of them being the true values at each of those new specific locations.
280 One way of computing the posterior predictive distributions for a whole region is to use
281 the unobserved vertices of a triangulation of the region as prediction locations, as
282 shown in Fig. 1. For the other points in the region of interest, the set of probable values
283 are obtained by use of additional interpolation tools.
284 Models were compared by considering two criteria: the Deviance Information Criterion
285 [30], usually denoted as DIC, which is computed routinely by INLA as the default
286 criterion for comparing hierarchical models, and the Conditional Predictive Ordinate
287 (CPO), which has been used as a predictive measure (of the models). In particular, as
288 indicate by Roos and Held [31], we computed the mean logarithmic CPO ($\overline{LCPO}$).
289 Lower values for both DIC and $\overline{LCPO}$ indicate better models.
290 The posterior means and first and third quartiles from the predictive distribution were
291 plotted to illustrate the predicted probability of occurrence of infection.
292
293 Results
294 The necropsies revealed that 111 out of the 589 (18.8%; 95% Bayesian Credible
295 Interval (BCI): 15.7%-21.9%) cows harboured Paramphistomidae flukes in their rumens
296 or reticula. The parasite burdens per animal ranged between 1 and 11895 (median=
297 266) flukes, and the parasites were located in much higher proportion in the rumen
298 (94.3% ± 14.1%) than in the reticulum (5.7% ± 8.3%). The distribution of parasites
299 within the rumen was also heterogeneous, with higher percentages found in the atrium
300 (58.2% ± 28.6%) and the rumenoreticular sulcus (26.5% ± 20.0%) than in the ventral
301 and dorsal sacs (9.5% ± 13.2% and 0.4% ± 1.1%, respectively).
302 Breakdown of the results of necropsies and coprological analyses by production type
303 (beef, milk) and age (<7 years, between 7-9 years, > 9 years) revealed that the

304 prevalence of infection was higher in the beef cows than in dairy cows (29.2%
305 compared with 13.9%), although with widely variable fluke burdens in both types of
306 animals (Table 1). In both groups, stool analysis also revealed high percentages of
307 infected animals excreting widely varying amounts of eggs. With regard to age, in cows
308 aged over 9 years, the prevalence of infection (25.0%), parasite burdens (range=5-
309 11895; median=351), percentage of animals with positive coprology, and faecal egg
310 counts (96.3 % cows excreting 1-2762 epg) were all higher than the younger cows. The
311 Bayesian GLM analysis (Table 2) showed that the effect of the type of production on
312 the prevalence of the infection, in particular the higher prevalence in beef cows, was
313 important as 95% BCI of the parameter for dairy cows clearly shifted towards negative
314 values. However, the type of production was not related to the fluke burdens, the
315 percentage of infected animals with positive coprology or the number of epg excreted
316 in faeces. The effect of age on the percentage of animals with positive coprology was
317 relevant, and the highest value was observed in cows over 9 years old (95% BCI of the
318 parameter shifted towards positive values). There was no relationship between the age
319 group and the prevalence of infection, the fluke burden or the number of epg. Finally,
320 there was a positive relation between the egg counts in the faeces and the parasite
321 burdens (posterior mean = 0.003; 95% CI = 0.002-0.004).
322 All the specimens examined were identified as *C. daubneyi*, on the basis of their
323 morphoanatomical characteristics. Moreover, the subsequent molecular analysis by
324 ITS-2 sequencing confirmed this specific classification, since the DNA amplification
325 revealed, in all specimens examined, a 410 bp fragment with a nucleotide composition
326 identical (100% homology) to that published for *C. daubneyi* in Gen BankTM
327 [GenBank: AY790883].
328 A total of 235 roe deer hunted in the vicinity of cattle farms distributed throughout
329 Galicia were examined by necropsy; no infections by paramphistomes were found in
330 any of them.

331 Not surprisingly, given the distribution of the cattle farms in Galicia (see Materials and
332 Methods 2.1), the dairy and beef farms sampled in this study were unevenly distributed
333 throughout the region (Fig. 2). Because of this and the large differences in the
334 prevalences of *C. daubneyi* infection in dairy and beef cows, we modelled the
335 probability of occurrence of this infection separately in the different types of cattle. The
336 model selected by the DIC and $\overline{LCPO}$ criteria for fitting to the data on *C. daubneyi*
337 infection in dairy cows included the annual mean temperature and the log-transformed
338 slope (for smoothing the effect and preserve the linearity of this variable), as
339 covariates, and a stochastic spatial component that accounted for the residual spatial
340 autocorrelation (Table 3). The annual mean temperature had a negative effect on the
341 occurrence of *C. daubneyi* infection, whereas the effect of the slope on the response
342 variable was positive and more important (in the sense that the posterior probability of
343 being different from zero was greater). The spatial component showed a strong effect,
344 with positive values in the centre of Galicia (specifically in the southern part of the
345 provinces of A Coruña and Lugo), and values around zero in the northern, western and
346 southern zones of the study area (Fig. 3, which depicts the posterior mean (A) and
347 standard deviation (B) of the spatial random effect).
348 The maps included in Fig. 4 show the mean predicted distribution of the probability of
349 infection of dairy cows across Galicia (A) and the uncertainty around this estimation
350 provided by the lower 25% (B) and upper 75% (C) quartiles of the posterior distribution
351 for the predicted probability of infection by *C. daubneyi*. It should be noted that because
352 of the small population of dairy cattle in the south and east of Galicia, very few cows
353 from these areas were included in the sample (see points overlaid on Fig. 4A), so that
354 predictions obtained for those locations should be interpreted with caution. Excluding
355 these areas, it can be seen that the the highest values of the probability of occurrence
356 of *C. daubneyi* infection covers the centre of Galicia, while the predicted probability for
357 the warmer and flatter western fringe is very low.

358 The results of inference for beef cows are shown in Table 4. In this case, the rainfall
359 and the density of cows in the municipality of origin were incorporated in the model as
360 covariates, and no spatial effect was detected. The probability of occurrence of
361 infection in beef cows is higher in the central part of Galicia, where the municipalities
362 with the highest densities of cattle are located (Fig. 5). The highest probabilities of
363 infection (up to 0.8) were predicted for some small zones scattered within this central
364 area, mainly in the farthest eastern zone, which is the driest zone within the region.
365
366 Discussion
367 This paper presents the results of a comprehensive study designed to investigate
368 different aspects of the epidemiology of bovine paramphistomosis in Galicia, the main
369 cattle-producing region in Spain. One interesting finding of the study is that infections
370 by paramphistomes are more prevalent among beef cows (29.2%) than among dairy
371 cows (13.9%), which probably reflects differences in exposure to infection, possibly
372 because of a closer association with pasture in the case of beef cows. This suggests
373 that the prevalence of parahimphistomosis has so far been underestimated in Galician
374 beef cattle. Earlier coprological surveys conducted in such animals had reported
375 prevalence rates of 10%-19% [32, 22, 23]. This discrepancy may be due to the different
376 origin of the animals examined. In the present study, prevalences were estimated from
377 data from animals on farms throughout Galicia, whereas only cattle from the northeast
378 of the region (province of Lugo) had previously been analyzed. Therefore, the higher
379 figure found in the present study may be a more accurate estimate of the true
380 prevalence of these infections in the whole region. Moreover, as demonstrated in the
381 present study, coprology does not detect all infections observed by necropsy.
382 Few studies have reported the size of the paramphistomes burdens present in naturally
383 infected cattle; however, the number of flukes in rumens and reticula determines the
384 degree of damage caused in the rumen and the potential detrimental effect on animal
385 health and production [4]. Most cows in the present study had low parasite burdens

386 (Median=266 flukes), similar to those previously reported by Arias et al. [33] for north-
387 west Spain and northern Portugal and by Szmidt-Adjidé et al. [34] for central France.
388 Nevertheless, we also found some animals harbouring large parasite burdens (up to
389 11895 flukes), which might cause production losses, particularly under conditions of
390 stress [9].
391 Stool analysis revealed that a high proportion of infected cows (83.8%) excreted
392 detectable amounts of paramphistomid eggs in their faeces. Specifically, 96.3% of
393 cows over 9 years shed fluke eggs, sometimes in large amounts (up to 2762 epg).
394 These results highlight the importance of adult cows, especially older cows, in
395 maintaining and transmitting paramphistomosis within cattle herds. Adult cows, which
396 constitute the bulk of animals in herds, spend long periods of their life on pastures,
397 depositing large volumes of faeces that contaminate snail habitats with numerous
398 parasite eggs. Therefore, mature cows are important reservoirs that transmit the
399 infection to the new generations of the susceptible animals entering the herd.
400 Identification of wild reservoir hosts for parasites is a prerequisite for the control of
401 parasitic diseases in endemic areas. However, to date no study had investigated the
402 occurrence of natural infections by paramphistomes in roe deer, which is the most
403 abundant wild ruminant in Galicia and which may act as a reservoir for
404 paramphistomosis [35, 36]. In the present study, paramphistomid flukes were not found
405 in any of the 235 roe deer examined by necropsy, which clearly demonstrates that the
406 role of this animal species in the current epidemiology of paramphistomosis in this
407 region is negligible. The apparent absence of wild reservoir hosts for this parasitosis in
408 Galicia underlines the importance of adult cows as reservoir for the infection in this
409 region.
410 Paramphistomosis may be caused by different genera and species of the family
411 Paramphistomidae, which are difficult to identify due to the morphological similarities
412 among them. Indeed, most reports of paramphistomosis do not specify the species that
413 cause the disease. Nevertheless, species might differ in important aspects such as the

414 range of susceptible hosts, pathogenicity, resistance to drugs and the geographical
415 distribution, among other factors. *Calicophoron daubneyi* appears to be the most
416 common paramphistomid affecting cattle in Europe [15, 37]. This was also the only
417 species reported by Díaz et al. [32, 22, 23] and Arias et al. [33] in Galicia, although
418 neither the number of specimens identified nor the method used for identification were
419 specified in these studies. In the present study, we used morpho-anatomical (n=618)
420 and molecular techniques (n=82) to examine a large number of fluke specimens
421 collected from various anatomical zones within the rumens and reticula of animals from
422 different areas of Galicia. This methodology enabled us to confirm that *C. daubneyi* is
423 the only species of Paramphistomidae parasitizing cattle in Galicia.
424 As with other trematodes, the transmission of *C. daubneyi* depends on the distribution
425 of the intermediate host snails involved in its life cycle. This distribution, as well as the
426 development and survival of the intra-molluscan and free-living stages of the parasite
427 are determined by climatic and other environmental conditions, which may vary
428 considerably among the different locations within wider geographic areas. Therefore,
429 the spatial distribution of infection risk is not homogeneous, and estimates of its
430 geographical distribution are needed to identify the locations where the risk of infection
431 is high and where monitoring and control interventions should be targeted. Over the
432 last decade, several studies have used satellite-derived environmental data to model
433 the occurrence and prevalence of animal helminthosis [38, 39, 40, 41]. Although a
434 useful benchmark for future applications, these approaches could be improved by
435 applying more flexible and robust spatial statistical methods, such as those based on
436 Bayesian approach. These methods offer advantages over those based on traditional
437 statistics since they enable the spatial correlation of the variables and the uncertainty of
438 the parameters to be included in the modelling process. Although Bayesian analysis
439 has been increasingly incorporated to the study of the geographical distribution of
440 human helminthosis in recent years [42, 43, 44, 45, 46, 47, 48], it has scarcely been

441 used in the context of animal diseases [49, 50, 51], probably because of the relative
442 complexity and computational difficulty of Bayesian modelling [42].
443 In the present study, spatial predictions of the probability of *C. daubneyi* infection
444 across Galicia were based on hierarchical Bayesian models developed separately for
445 dairy and beef cattle. The best models (in terms of DIC and $\overline{LCPO}$) suggested that
446 different covariates predicted the probability of infection in each type of cattle. The
447 spatial pattern in dairy cattle was mainly governed by temperature and slope in the
448 areas where the farms were located, so that the probability of infection increased with a
449 decreasing mean temperature and increasing slope. However, in beef cattle, the
450 annual rainfall and density of cows were the main predictive covariates, and infections
451 were more likely to occur in locations where the rainfall was lower and the stocking rate
452 was higher. The positive relationship between density of cows and probability of
453 infection was consistent with the general theoretical knowledge about pasture-
454 transmitted parasitosis. However, different studies have demonstrated that the effects
455 of environmental covariates on the occurrence and/or prevalence of trematode
456 infections are not so obvious. In this regard, Raspch et al. [52] indicated that in the
457 case of *Fasciola hepatica*, a fluke that has the same snail as an intermediate host, the
458 risk of infection increases with increasing rainfall, until reaching a maximum at
459 approximately 90 mm per month; however, large amounts of rainfall (over 210 mm per
460 month) can wash parasite larvae and snails away and separate them from each other,
461 thus inhibiting transmission. Subsequently, Bennema et al. [53] suggested that the
462 threshold for such a “wash away” effect may be considerably lower, since they found a
463 negative association between rainfall and presence of economically important *F.*
464 *hepatica* infections in a region of Belgium (Flanders) where the average values for
465 annual and monthly rainfall were 800 mm and 61.1 mm, respectively. The present
466 results in a zone with a similar mean annual precipitation (1065 mm) appear to confirm
467 this suggestion. As regards temperature, it is known that, under laboratory conditions
468 (i.e., with sufficient moisture) the development rate of the fluke larval stages increases

between 10 ℃ and 25 ℃ [54], so that temperatures within this range are also expected to be a risk factor for infection of the ruminants. However, under field conditions, the relationship between risk of infection and temperature is more complex, largely due to the strong interactions between air temperature, moisture content of ground and soil permeability. The negative association between temperature and probability of infection in the present study suggests that the suitable thermal range for transmission of infection in Galicia is narrower than expected, probably because most soils in this region are highly permeable and dry out quickly in summer, when the temperature increases in the absence of rainfall. Therefore, in the warmest locations, interruption of the emission of cercariae and shortening of survival of metacercariae will occur during summer, with the subsequent reduction in the intensity of infection transmission. In the present study, slope was positively associated with the probability of infection. This is consistent with the findings reported by Cringoli et al. [55] for *C. daubneyi* and by Bennema et al. [53] for *F. hepatica* infections, which the authors attributed to the ponding (temporary or permanent) that typically occurs in the lower areas of sloping pastures in rainy locations. In contrast, McCann et al. [56] found a negative effect of the slope on the prevalence of *F. hepatica* infection, which they attributed to better drainage. In a study carried in north-eastern Galicia, Díaz et al. [22] also found a negative effect of slope on the prevalence of *C. daubneyi* infection, although only when the slope was very steep (exceeding 25%), so that the previously observed discrepancies may be due to differences in the ranges of slope values in the different studies. However, the results of most previous studies should be regarded with caution due to the unsuitability of the applied statistical tests (chi-square test, Spearman rank order correlation and linear regression), which may have led to biased parameter estimates as spatial correlation was ignored.

There were two important differences in the models fitted for the probability of *C. daubneyi* infection in dairy and beef cattle. First, different environmental covariates were used to explain the probability of the infection in both models. This may reflect

497 variations in the effects of environmental covariates depending on the range of values
498 and potential interactions among them in the different areas where the farms of origin
499 of both types of animals were located. In this sense, it must be taken into account the
500 dairy and beef farms were unevenly distributed throughout the study area (see
501 Materials and Methods) and that values for covariates at the locations of dairy and beef
502 farms differed significantly (data not shown). The second difference is related to the
503 residual spatial correlation, which existed in the model of infection transmission of dairy
504 cows, but not in that of beef cows. The overall transmission success depends on the
505 establishment of the parasite in the ruminant host, which in turn is determined by the
506 ingestion of infective metacercariae during grazing. Since beef cows in Galicia graze
507 throughout the year and throughout their lives, infection is guaranteed when the grass
508 is infected with metacercariae. Therefore, variations in the risk of infection of beef cattle
509 will be primarily determined by the environmental covariates that determine
510 development of the parasite, its intermediate host, and ultimately the presence of
511 infective metacercariae on the grass. However, the grazing patterns of dairy cows are
512 different; they may graze for their whole life or only during some stages (heifers, dry
513 cows), throughout the whole year or only seasonally, or they may even never graze on
514 pasture. This implies great differences as regards the possibilities of contact with
515 metacercariae and in the actual risk of infection. As we could not include this
516 information in the modelling process, the residual autocorrelation of the infection in
517 dairy cattle may reflect the risk of infection associated with the differences in the
518 grazing history of the animals.
519 This study has provided the first maps of the spatial distribution of the probability of *C.*
520 *daubneyi* infection in beef and dairy cattle. Its use allowed us to identify the centre of
521 Galicia as the zone with the highest risk of infection for both types of cattle. This is also
522 the zone with the largest livestock population, so that it should be the preferential target
523 for campaigns to raise the awareness of farmers and veterinarians about the situation
524 and for monitoring interventions. Nevertheless, in the case of dairy cows, the estimates

525 may be inaccurate because of the lack of information about grazing patterns and other
526 management factors which were not measured in this study. For example, de-worming
527 treatments will affect the establishment, survival and fecundity of adult worms, and
528 therefore will also affect the transmission patterns of the infection. Consequently, the
529 next logical step is to include such information to further refine the model and enhance
530 the accuracy of prediction. Furthermore, we believe that the use of this approach for
531 constructing maps of the spatial distribution of infections and co-infections by other
532 common helminths in the region, such as those produced by *F. hepatica* and
533 gastrointestinal nematodes, may help design integrated programs for more efficient
534 surveillance and control of bovine helminthoses.
535
536 ## Competing interests
537 The authors declare that they have no competing interests.
538
539 ## Authors' contributions
540 Conception and design of the study: MGW and MM. Sample and data collection: MGW,
541 JACH and MM. Species identification by microscopy and molecular techniques: AMMI
542 and YMG. Bayesian statistical analysis: SL, DC, FM, ALQ. Manuscript draft
543 preparation: MGW, JACH, DC, YMG and MM. All authors have read and approved the
544 final manuscript.
545
546 ## Acknowledgements
547 This study was supported by grant FAU2006-00021-C03-00 from the Ministerio de
548 Economía y Competitividad. The authors thank the personnel of the Servizo de
549 Sanidade Animal de la Consellería do Medio Rural e do Mar (Xunta de Galicia) for their
550 helpful collaboration in collecting samples from deer and the staff of the Servizo
551 Cartográfico de Galicia (SITGA) for providing data on environmental variables. The
552 authors are also grateful to Dr. González-Lanza, C. Espiniella and M.P. Del-Pozo, all

members of the CSIC staff at the “Instituto de Ganadería de Montaña (CSIC-ULE), León, Spain. A.M. Martínez-Ibeas is supported by the “Castile and León” Autonomy (Spain) and the European Social Funds (Contract for young researchers).

References

1. Jones A: Family Paramphistomidae Fischoeder, 1901. In *Keys to the Trematoda, Volume 2*. Edited by Jones A, Bray RA, Gibson DI. CAB International and the Natural History Museum, London; 2005:229-246.

2. Singh RP, Sahai BN, Jha GJ: Histopathology of the duodenum and rumen of goats during experimental infections with *Paramphistomum cervi. Vet Parasitol* 1984, 15:39-46.

3. Dorchies P, Lacroux C, Navetat H, Rizet C, Guéneau E, Bisson B, Ferté H: Trois cas d’une nouvelle entité pathologique: la paramphistomose larvaire chez les bovins. *Bull GTV* 2002, 13:91-93.

4. Mavenyengwa M, Mukaratirwa S, Obwolo M, Monrad J: A macro- and light microscopical study of the pathology of *Calicophoron microbothium* infection in experimentally infected cattle. *J Vet Res* 2005, 72:321-332.

5. Rolfe RP, Boray JC, Collins GH: Pathology of infection with *Paramphistomum ichikawai* in sheep. *Int J Parasitol* 1994, 24:995-1004.

6. Rolfe RP, Boray JC, Nichols P, Collins GH: Epidemiology of paramphistomosis in cattle. *Int J Parasitol* 1991, 21:813-819.

580 7. Mavenyengwa M, Mukaratirwa S, Monrad J: Influence of *Calicophoron*
581 *microbothrium* amphistomosis on the biochemical and blood cell counts of
582 cattle. *J. Helminthol*. 2010, 84:355-361.
583
584 8. Dorny P, Stoliaroff V, Charlier J, Meas S, Sorn S, Chea B, Holl D, Van Aken D,
585 Vercruysse J: Infections with gastrointestinal nematodes, Fasciola and
586 Paramphistomum in cattle in Cambodia and their association with morbidity
587 parameters. *Vet Parasitol* 2011, 10:293-299.
588
589 9. Spence SA, Fraser GC, Dettmann EB, Battese DF: Production responses to
590 internal parasite control in dairy cattle. *Aust Vet J* 1992, 69:217-220.
591
592 10. Spence SA, Fraser GC, Chang S: Responses in milk production to control of
593 gastrointestinal nematode and paramphistome parasites in dairy cattle. *Aust Vet*
594 *J* 1996, 74:456-459.
595
596 11. Mavenyengwa M, Mukaratirwa S, Obwolo M, Monrad J: Bovine intestinal cellular
597 responses following primary and challenge infections with *Calicophoron*
598 *microbothrium* metacercariae. *Onderstepoort J Vet Res* 2008, 75:109-120.
599
600 12. Rangel-Ruiz LJ, Albores-Brahms ST, Gamboa-Aguilar J: Seasonal trends of
601 *Paramphistomum cervi* in Tabasco, Mexico. *Vet Parasitol* 2003, 116:217-222.
602
603 13. Phiri AM, Chota A, Phiri IK: Seasonal pattern of bovine amphistomosis in
604 traditionally reared cattle in the Kafue and Zambezi catchment areas of Zambia.
605 *Trop Anim Health Prod* 2007, 39:97-102.
606

14. Rieu E, Recca A, Bénet JJ, Saana M, Dorchies P, Guillot J: Reliability of coprological diagnosis of *Paramphistomum* sp. infection in cows. *Vet Parasitol* 2007, 31:249-253.

15. Mage C, Bourgne H, Toulieu JM, Rondelaud D, Dreyfuss G: *Fasciola hepatica* and *Paramphistomum daubneyi*: changes in prevalences of natural infections in cattle and in *Lymnaea truncatula* from central France over the past 12 years. *Vet Res* 2002, 33:439-447.

16. Dinnik JA: *Paramphistomum daubneyi* sp. nov. from cattle and its snail host in the Kenya Highlands. *Parasitology* 1962, 52:143-151.

17. Eduardo SL: The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of species occurring in ruminants. III. Revision of the genus *Calicophoron* Näsmark, 1937. *Systematic Parasitology* 1983, 5:25-79.

18. Foster AP, Otter A, O'Sullivan T, Cranwell MP, Twomey DF, Millar MF, Taylor MA: Rumen fluke (paramphistomosis) in British cattle. *Vet Rec* 2008, 162:528.

19. Murphy TM, Power EP, Sanchez-Miguel C, Casey MJ, Toolan DP, Fagan JG: Paramphistomosis in Irish cattle. *Vet Rec* 2008, 162:831.

20. Cordero M, Castañón L, Reguera A: Índice catálogo de zooparásitos ibéricos. 2nd editcion. Secretariado de Publicaciones. Universidad de León, León (Spain), 1994, 650 pp.

21. Ramajo-Martín V, Pérez-Sánchez R, Ramajo-Hernández A, Oleaga A: Preliminary data about the parasitism caused by Protozoa, Helminths and Ticks in cervids and wild bovids from Salamanca (western Spain). *Rev Ibér Parasitol* 2007, 67:69-77.

22. Díaz P, Paz-Silva A, Sánchez-Andrade R, Suárez JL, Pedreira J, Arias M, Díez-Baños P, Morrondo P: Assessment of climatic and orographic conditions on the infection by *Calicophoron daubneyi* and *Dicrocoelium dendriticum* in grazing beef cattle (NW Spain). *Vet Parasitol* 2007a, 149:285-289.

23. Díaz P, Pedreira J, Sánchez-Andrade R, Suárez JL, Arias MS, Francisco I, Fernández G, Díez-Baños P, Morrondo P, Paz-Silva A: Risk periods of infection by *Calicophoron daubneyi* (Digenea: Paramphistomidae) in cattle from oceanic climate areas. *Parasitol Res* 2007b, 101:339-342.

24. Rue H, Martino S, Chopin N: Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *J R Stat Soc Series B* 2009, 71:319-392.

25. Martínez-Ibeas AM, González-Warleta M, Mezo M, Martínez-Valladares M, González-Lanza C, Miñambres B, Castro-Hermida JA, Pérez V, Ferreras-Estrada C, Manga-González MY: Preliminary study of the genetic variability of *Calicophoron daubneyi* (Paramphistomidae) in cattle in North-West Spain. In *Proceedings of the XII Congreso Ibérico de Parasitología; Zaragoza (Spain).* 2011:242.

26. Banerjee S, Carlin B, Gelfand A: Hierarchical Modeling and Analysis for Spatial Data. Edited by Chapman and Hall/CRS Press. 2004: 472 pp.

662 27. Gelfand AE, Ravishanker N, Ecker MD: Modeling and inference for point-
663 referenced binary spatial data. In *Generalized Linear Models: A Bayesian*
664 *Perspective*. Edited by Dey D, Ghosh S and Mallick B: Marcel Dekker Inc; 2000:381-
665 394.
666
667 28. Diggle PJ, Tawn JA, Moyeed RA: Model-based geostatistics. *J R Stat Soc,*
668 *Series C (Applied Statistics)* 1998, 47:299-350.
669
670 29. Lindgren F, Rue H, Lindström J: 2011. An explicit link between Gaussian fields
671 and Gaussian Markov random fields: the SPDE approach (with discussion). *J R*
672 *Stat Soc, Series B* 2011, 73:423-498.
673
674 30. Spiegelhalter DJ, Best NG, Carlin BP, van der Linde A: Bayesian measures of
675 model complexity and fit. *J R Stat Soc, Series B* 2002, 64:583-616.
676
677 31. Roos M, Held L: Sensitivity analysis in Bayesian generalized linear mixed
678 models for binary data. *Bayesian Analysis* 2011, 6:259-278.
679
680 32. Díaz P, Lomba C, Pedreira J, Arias M, Sánchez-Andrade R, Suárez JL, Díez-
681 Baños P, Morrondo P, Paz-Silva A: Analysis of the IgG antibody response against
682 Paramphistomidae trematoda in naturally infected cattle. Application to
683 serological surveys. *Vet Parasitol* 2006, 140:281-288.
684
685 33. Arias M, Lomba C, Dacal V, Vázquez L, Pedreira J, Francisco I, Piñeiro P,
686 Cazapal-Monteiro C, Suárez JL, Díez-Baños P, Morrondo P, Sánchez-Andrade R, Paz-
687 Silva A: Prevalence of mixed trematode infections in an abattoir receiving cattle
688 from nothern Portugal and north-west Spain. *Vet Rec* 2011, 168:408-412.
689

690 34. Szmidt-Adjidé V, Abrous M, Adjidé CC, Dreyfuss G, Lecompte A, Cabaret J,
691 Rondelaud D: Prevalence of *Paramphistomum daubneyi* infection in cattle in
692 central France. *Vet Parasitol* 2000, 87:133-138.
693
694 35. Páv J, Zajícek D, Dvorák M: Clinical examination of the blood of roe deer
695 (*Capreolus capreolus* L.) and fallow deer (*Dama dama* L.) naturally invaded by
696 parasites. *Vet Med (Praha)* 1975, 20:215-221.
697
698 36. Pavlovic I, Savic B, Ivanovic S, Cirovic D: First occurrence of *Paramphistomum*
699 *microbothrium* (Fischoeder 1901) in roe deer (*Capreolus capreolus*) in Serbia. *J*
700 *Wildl Dis* 2012, 48:520-522.
701
702 37. Rinaldi L, Perugini AG, Capuano F, Fenizia D, Musella V, Veneziano V, Cringoli G:
703 Characterization of the second internal transcribed spacer of ribosomal DNA of
704 *Calicophoron daubneyi* from various hosts and locations in southern Italy. *Vet*
705 *Parasitol* 2005, 131:247-253.
706
707 38. Tum S, Puotinen ML, Copeman DB: A geographic information system model for
708 mapping risk of fasciolosis in cattle and buffaloes in Cambodia. *Vet Parasitol*
709 2004, 122:141-149.
710
711 39. Tum S, Puotinen ML, Skerratt LF, Chan B, Sothoeun S: Validation of a
712 geographic information system model for mapping the risk of fasciolosis in
713 cattle and buffaloes in Cambodia. *Vet Parasitol* 2007, 143:364-367.
714
715 40. Bennema S, Vercruysse J, Morgan E, Stafford K, Höglund J, Demeler J, von
716 Samson-Himmelstjerna G, Charlier J: Epidemiology and risk factors for exposure

717 to gastrointestinal nematodes in dairy herds in northwestern Europe. *Vet*
718 *Parasitol* 2010, 173:247-254.
719
720 41. McCann CM, Baylis M, Williams DJL: Seroprevalence and spatial distribution of
721 *Fasciola hepatica*-infected dairy herds in England and Wales. *Vet Rec* 2010a,
722 166:612-617.
723
724 42. Clements CA, Lwambo JS, Blair L, Nyandini U, Kaatano G, Kinung'hi S, Webster
725 JP, Fenwick A, Brooker S: Bayesian spatial analysis and disease mapping: tools to
726 enhance planning and implementation of a schistosomiasis control programme
727 in Tanzania. *Trop Med Int Health* 2006, 11:490-503.
728
729 43. Clements CA, Garba A, Sacko M, Touré S, Dembelé R, Landouré A, Bosque-Oliva
730 E, Gabrielli AF, Fenwick A: Mapping the probability of Schistosomiasis and
731 associated uncertainty, West Africa. *Emerg Infect Dis* 2008, 14:1629-1632.
732
733 44. Raso G, Vounatsou P, Gosoniu L, Tanner M, N'Goran EK, Utzinger J: Risk factors
734 and spatial patterns of hookworm infection among schoolchildren in a rural area
735 of western Côte d'Ivoire. *Int J Parasitol* 2006, 36:201-210.
736
737 45. Brooker S, Clements AC: Spatial heterogeneity of parasite co-infection:
738 Determinants and geostatistical prediction at regional scales. *Int J Parasitol* 2009,
739 39:591-597.
740
741 46. Simoonga C, Utzinger J, Brooker S, Vounatsou P, Appleton CC, Stensgaard AS,
742 Olsen A, Kristensen TK: Remote sensing, geographical information system and
743 spatial analysis for schistosomiasis epidemiology and ecology in Africa.
744 *Parasitology* 2009, 136:1683-1693.

745

746 47. Schur N, Hürlimann E, Stensgaard AS, Chimfwembe K, Mushinge G, Simoonga C,
747 Kabatereine NB, Kristensen TK, Utzinger J, Vounatsou P: Spatially explicit
748 Schistosoma infection risk in eastern Africa using Bayesian geostatistical
749 modelling. *Acta Trop*, in press.

750

751 48. Soares-Magalhaes RJ, Clements ACA, Patil AP, Gething PW, Brooker S: The
752 application of model-based geostatistics in helminth epidemiology and control.
753 *Adv Parasitol* 2011, 74:267-296.

754

755 49. Durr PA, Tait N, Lawson AB: Bayesian hierarchical modelling to enhance the
756 epidemiological value of abattoir surveys for bovine fasciolosis. *Prev Vet Med*
757 2005, 71:157-172.

758

759 50. Allepuz A, López-Quílez A, Forte A, Fernández G, Casal J: Spatial analysis of
760 bovine spongiform encephalopathy in Galicia. *Prev Vet Me*d 2007, 79:174-185.

761

762 51. Musella V, Catelan D, Rinaldi L, Lagazio C, Cringoli G, Biggeri A: Covariate
763 selection in multivariate spatial analysis of ovine parasitic infection. *Prev Vet Med*
764 2010, 99:69-77.

765

766 52. Raspch C, Dahinden T, Heinzmann D, Torgerson PR, Braun U, Deplazes P, Hurni
767 L, Bär H, Knubben-Schweizer G: An interactive map to assess the potential spread
768 of *Lymnaea truncatula* and the free-living stages of *Fasciola hepatica* in
769 Switzerland. *Vet Parasitol* 2008, 154, 242-249.

770

771 53. Bennema S, Ducheyne E, Vercruysse J, Claerebout E, Hendrickx G, Charlier J:
772 Relative importance of management, meteorological and environmental factors

773 in the spatial distribution of *Fasciola hepatica* in dairy cattle in a temperate
774 climate zone. *Int J Parasitol* 2011, 41:225-233.
775
776 54. Andrews SJ: The life cycle of *Fasciola hepatica*. In: Fasciolosis. Edited by Dalton
777 JP, Oxon: CABI; 1999:1-29.
778
779 55. Cringoli G, Taddei R, Rinaldi L, Veneziano V, Musella V, Cascone C, Sibilio G,
780 Malone JB: 2004. Use of remote sensing and geographical information systems to
781 identify environmental features that influence the distribution of
782 paramphistomosis in sheep from the southern Italian Apennines. *Vet Parasitol*
783 2004, 122:15-26.
784
785 56. McCann CM, Baylis M, Williams DJL: The development of linear regression
786 models using environmental variables to explain the spatial distribution of
787 *Fasciola hepatica* infection in dairy herds in England and Wales. *Int J Parasitol*
788 2010b, 40:1021-1028.
789

790 Figure 1. Triangulation map covering the region. Each mesh vertex is either an
791 observed point (●) or a prediction point.
792
793 Figure 2. Geografical distribution of the farms of origin of the dairy cows (A) and
794 beef cows (B) sampled in the slaughterhouse. Cows infected by *C. daubneyi* (•)
795 and uninfected cows (•).
796
797 Figure 3. Spatial component of the fitted model for the probability of occurrence
798 of *Calicophoron daubneyi* infection in dairy cows throughout Galicia. Posterior
799 mean (A) and standard deviation (B).
800
801 Figure 4. Predicted probability of the occurrence of *C. daubneyi* infection in
802 dairy cows throughout Galicia. Mean (A), $Q_{0.25}$ (B) and $Q_{0.75}$ (C) of the posterior
803 distribution. The overlaid points mark the geographical origin of the cows in the
804 sample, distinguishing between cows infected by *C. daubneyi* (•) and uninfected
805 cows (•).
806
807 Figure 5. Predicted probability of the occurrence of *C. daubneyi* infection in beef
808 cows throughout Galicia. Mean (A), $Q_{0.25}$ (B) and $Q_{0.75}$ (C) of the posterior
809 distribution. The overlaid points mark the geographical origin of the cows in the
810 sample, distinguishing between cows infected by *C. daubneyi* (•) and uninfected
811 cows (•).
812

Table 1 Prevalence and intensity of infections by paramphistomes in cows.

	Necropsy			Coprology	
	N	Prevalence (%)	Fluke burden Range (median)	Positive[1] (%)	epg Range (median)
Type of production					
Beef	192	29.2	3-11070 (257)	89.3	1-855 (16)
Milk	397	13.9	1-11895 (307)	78.2	1-2762 (31)
Age (years)					
<7	200	14.5	4-6009 (305)	79.3	2-1100 (18)
7-9	173	16.2	1-4664 (165)	64.3	3-573 (19)
>9	216	25.0	5-11895 (351)	96.3	1-2762 (25)
Total	589	18.8	1-11895 (266)	83.8	1-2762 (22)

[1] Percentage calculated on the basis of the number of animals with infection by paramphistomes detected by necropsy

Table 2 Bayesian GLM and LM analysis of results obtained by necropsy and coprological analysis.

	Posterior distribution means (95% BCI)[1]			
	Prevalence	Fluke burden	Positive coprology	epg
Intercept including reference group				
Beef and <7 years	-1.13 (-1.67, -0.61)	5.33 (4.35, 6.30)	1.57 (0.41, 2.89)	0.88 (0.84, 0.92)
Type of production				
Milk	-0.83 (-1.30, -0.36)	0.26 (-0.59, 1.11)	-0.29 (-1.48, 0.85)	-0.01 (-0.05, 0.02)
Age (years)				
7-9	0.12 (-0.45, 0.69)	-0.85 (-1.96, 0.26)	-0.80 (-2.02, 0.36)	0.03 (-0.02, 0.07)
>9	0.33 (-0.21, 0.88)	0.35 (-0.67, 1.36)	1.84 (0.25, 3.70)	-0.03 (-0.08, 0.01)
DIC[2]	557.76	489.24	90.67	-220.43

[1] BCI: Bayesian credible interval; [2] DIC: Deviance information criterion

Table 3 Bayesian spatial logistic regression model for the probability of infection of dairy cows with *C. daubneyi*.

	Summary of the posterior distributions[1]				
	Mean	SD	$Q_{0.025}$	$Q_{0.5}$	$Q_{0.975}$
(Intercept)	1.259	2.587	-3.852	1.270	6.308
Temperature	-0.398	0.233	-0.853	-0.398	0.061
Slope (Log)	0.517	0.228	0.082	0.512	0.979

DIC=305.04; LCPO=0.3849

[1]Coefficients were calculated taking into account the geospatial effect.

Table 4 Bayesian spatial logistic regression model for the probability of infection of beef cows with *C. daubneyi*.

	Summary of the posterior distributions				
	Mean	SD	$Q_{0.025}$	$Q_{0.5}$	$Q_{0.975}$
(Intercept)	0.510	1.183	-1.791	0.503	2.850
Rainfall	-0.003	0.001	-0.005	-0.003	-0.000
Cattle density	0.040	0.009	0.022	0.039	0.058

DIC=209.96; LCPO=0.55

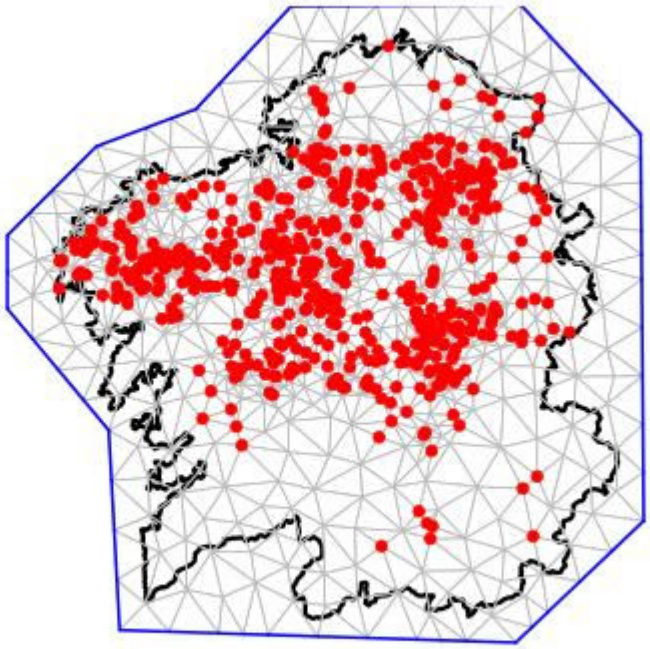
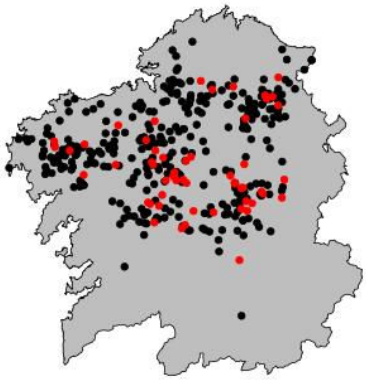


Figure 1

A)



B)

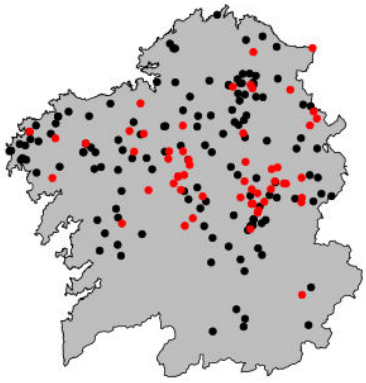
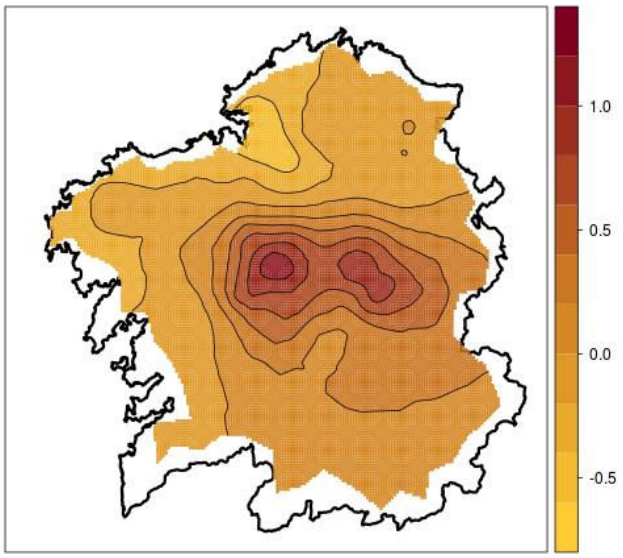


Figure 2

A)



B)

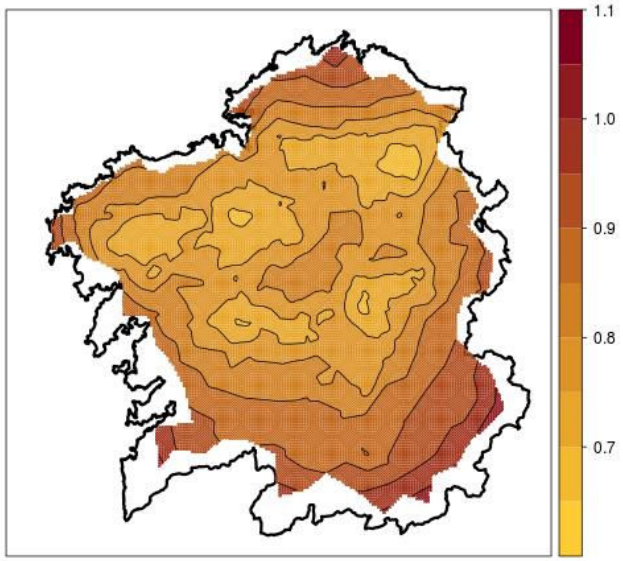
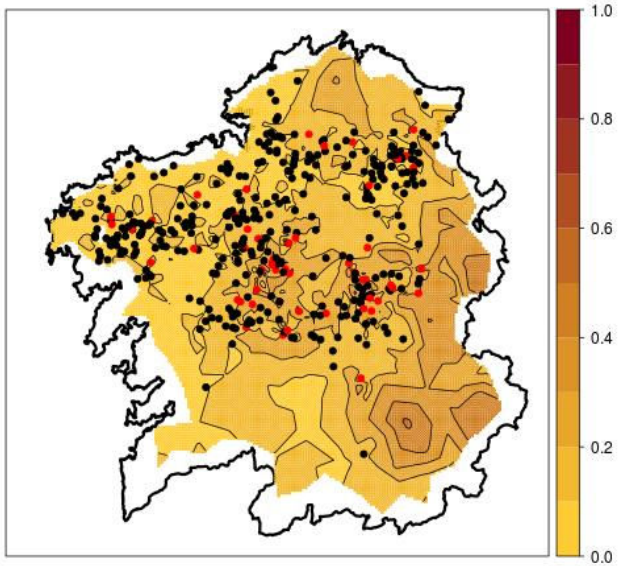


Figure 3

A)



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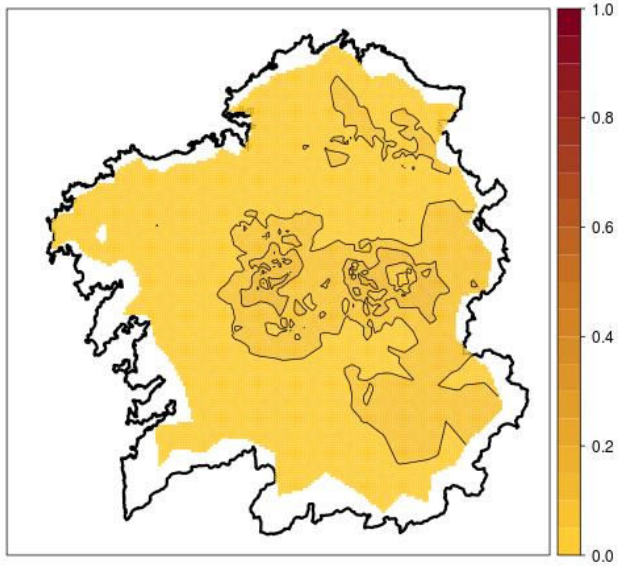


C)

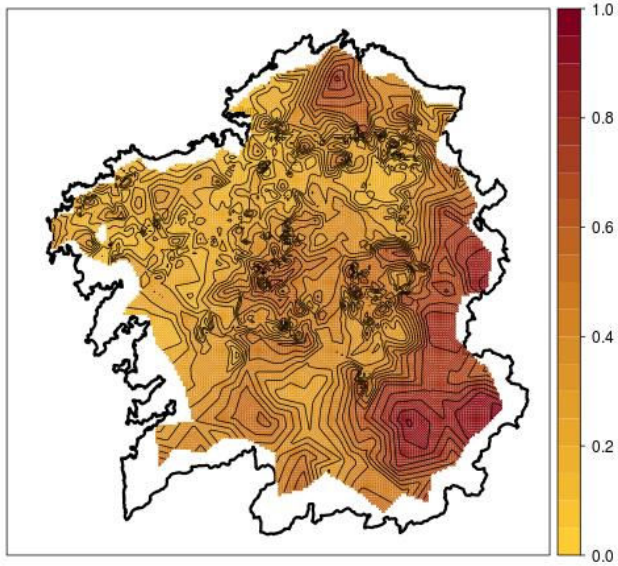
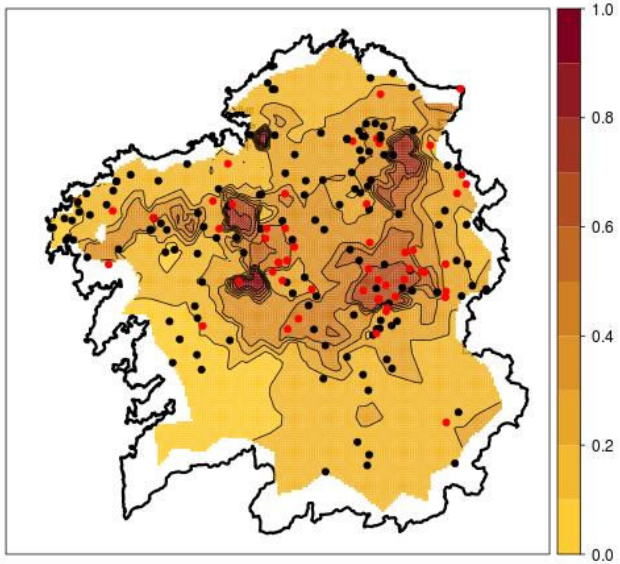
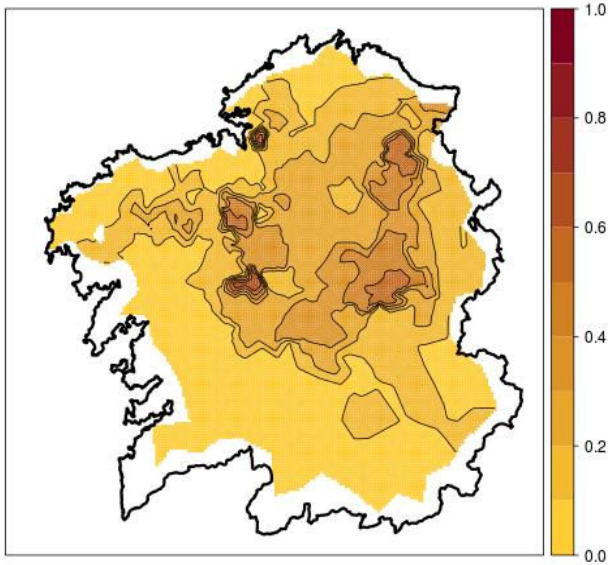


Figure 4

A)



B)



C)

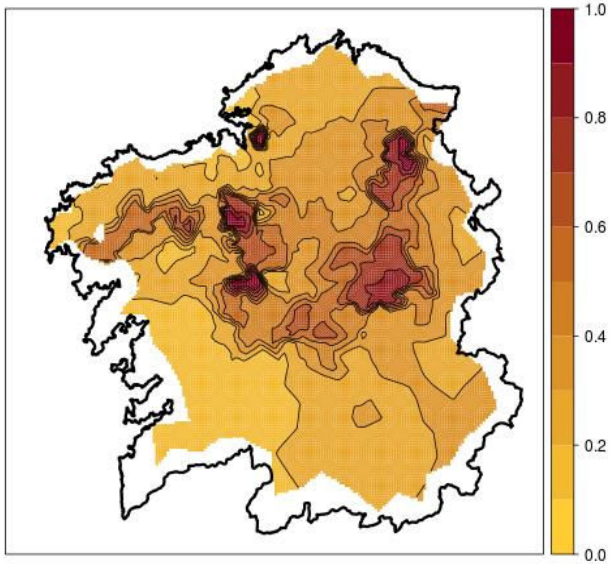


Figure 5