



Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: <http://www.tandfonline.com/loi/tjas20>

Prevalence and distribution of infectious and parasitic agents in roe deer from Spain and their possible role as reservoirs

María Patrocinio Morrondo, Ana Pérez-Creo, Alberto Prieto, Eva Cabanelas, José M. Díaz-Cao, María Sol Arias, Pablo Díaz Fernández, Gerardo Pajares, Susana Remesar, Ceferino M. López-Sández, Gonzalo Fernández, Pablo Díez-Baños & Rosario Panadero

To cite this article: María Patrocinio Morrondo, Ana Pérez-Creo, Alberto Prieto, Eva Cabanelas, José M. Díaz-Cao, María Sol Arias, Pablo Díaz Fernández, Gerardo Pajares, Susana Remesar, Ceferino M. López-Sández, Gonzalo Fernández, Pablo Díez-Baños & Rosario Panadero (2017) Prevalence and distribution of infectious and parasitic agents in roe deer from Spain and their possible role as reservoirs, Italian Journal of Animal Science, 16:2, 266-274, DOI: [10.1080/1828051X.2016.1245593](https://doi.org/10.1080/1828051X.2016.1245593)

To link to this article: <http://dx.doi.org/10.1080/1828051X.2016.1245593>



© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 16 Nov 2016.



[Submit your article to this journal](#)



Article views: 284



[View related articles](#)



[View Crossmark data](#)

Full Terms & Conditions of access and use can be found at <http://www.tandfonline.com/action/journalInformation?journalCode=tjas20>

Prevalence and distribution of infectious and parasitic agents in roe deer from Spain and their possible role as reservoirs

María Patrocínio Morrondo, Ana Pérez-Creo, Alberto Prieto, Eva Cabanelas, José M. Díaz-Cao, María Sol Arias, Pablo Díaz Fernández, Gerardo Pajares, Susana Remesar, Ceferino M. López-Sández, Gonzalo Fernández, Pablo Díez-Baños and Rosario Panadero

Departamento de Patología Animal (Grupo INVESAGA), Santiago of Compostela University, Lugo, Spain

ABSTRACT

In order to provide up-to-date information about the prevalence of infectious and parasitic agents in Spanish roe deer (*Capreolus capreolus*), samples from 93 animals hunted from January 2013 to April 2015 were collected and analysed by parasitological, serological and molecular techniques. Sampled animals came from four roe deer populations corresponding to Oceanic, Continental, Mediterranean and Mountainous ecosystems of Spain. Data regarding sex, age and year were also considered. A high percentage of roe deer (95.7%) resulted positive for at least one agent. *Sarcocystis* spp. was the most frequently diagnosed genus (88.8%), followed by gastrointestinal nematodes (62.9%) and Schmallenberg virus (53.5%). *Varestrongylus capreoli* (38%), *Anaplasma phagocitophilum* (34.2%), *Eimeria* spp. (29.2%), *Toxoplasma gondii* (25%) and *Cephenemyia stimulator* (23.8%) displayed medium prevalences and, finally low percentages were registered for *Moniezia* spp. (6.7%), *Dictyocaulus noeneri* (2.4%) and *Mycobacterium avium* subsp. *paratuberculosis* (1.5%). No infections by *Neospora caninum*, Bovine herpesvirus, pestivirus or *Coxiella burnetii* were found. Climate was significantly associated with the prevalence of *T. gondii*, *C. stimulator* and *A. phagocitophilum*, with higher prevalences in animals from Oceanic and Mediterranean areas. Our results suggest that infections affecting Spanish roe deer, especially those of parasitic aetiology, represent one of the causes of the descent in the abundance of this ungulate in the last years. Moreover, the high prevalence of zoonotic agents such as *T. gondii* and *A. phagocitophilum* could also have a great relevance in the environmental and/or Public Health.

ARTICLE HISTORY

Received 26 April 2016
Revised 1 October 2016
Accepted 4 October 2016

KEYWORDS

Bacteria; parasites; roe deer; Spain; viruses


Introduction

The roe deer (*Capreolus capreolus*, L.) is one of the most abundant wild ungulates in Spain, with a population around 600,000 animals (Carranza 2010). Spanish roe deer population is genetically heterogeneous; recent studies in Spain have revealed high molecular differentiation among Northwestern and Central-Southern roe deer (Royo et al. 2007).

Over the past few decades, wild ungulates have experienced an expansion throughout Europe both in the number and distribution. An increment in roe deer densities was also observed between 2000 and 2010 in the Iberian Peninsula (Fandos & Burón 2013; Valente et al. 2014). Forest regeneration due to rural exodus and hunting restrictions were considered as the main drivers of the expansion experienced by this ungulate. However, in the last 5 years, it has been recorded a

decrease in the abundance, especially in Northwestern Spain (Galicia & Asturias). Different causes have been postulated, including diseases, predation and starvation by severe weather conditions.

Parasites and infectious agents are an integral part of any natural ecosystem and communities. From an epidemiological point of view, wild animals can act as reservoirs and permanent source from which domestic animals and humans may be infected and invaded (Corner 2006). Recent studies carried out in the north-west of Spain have revealed a high prevalence of *Sarcocystis* spp., especially in the cardiac muscle (Pérez-Creo et al. 2013), along with a high occurrence of respiratory parasites such as *Dictyocaulus noeneri* (= *D. eckerti*) and *Varestrongylus capreoli* (Panadero et al. 2001; Morrondo et al. 2010) and the throat botfly *Cephenemyia stimulator* (Arias et al. 2014). The high

CONTACT Prof. Rosario Panadero  rosario.panadero@usc.es  Departamento de Patología Animal (Grupo INVESAGA), Santiago of Compostela University, Lugo, Spain

© 2016 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

incidence of cardio respiratory parasites could make roe deer an easy prey for predators, contributing to the dissemination of the parasites.

Alternative hypothesis to the lately decrease of roe deer populations include lower roe deer reproduction. *Toxoplasma gondii* and *Neospora caninum* are responsible for important reproductive failure in livestock (Dubey 1994, 1999). In this way, wild ruminants are suggested to act as intermediate hosts for both agents but the information currently available is still insufficient. Similarly, Schmallenberg virus (SBV) infection is commonly subclinical in ruminants; although its specific effects are still unknown, it may cause reproductive losses in pregnant animals (van den Brom et al. 2012).

Schmallenberg disease and Anaplasmosis are arthropod-borne illnesses, transmitted by *Culicoides* midges and ticks, respectively, which can affect both wild and domestic ruminants. *Anaplasma phagocytophilum* is responsible for tick-borne fever or Pasture fever in ruminants; it also causes haemorrhage, renal failure and neurological problems in infected humans (Stuen et al. 2013). Thus, its zoonotic role should also be considered, especially in immune compromised patients.

Ruminant Pestiviruses and Herpesviruses are worldwide distributed infections in domestic ruminants that cause severe reproductive disorders such as abortion and infertility problems. The presence of Pestiviruses and Herpesviruses has been also reported in wild ungulates, including roe deer (Vilcek & Nettleton 2006; Boadella et al. 2010); nevertheless, information about the epidemiology and risk factors of these viruses in roe deer is still scarce.

This study was designed to determine the prevalence of different agents affecting the cardio-respiratory (*Sarcocystis*, *C. stimulator*; lungworms and herpesvirus); digestive (*Eimeria* spp, *Moniezia* spp, gastrointestinal (GI) nematodes, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and pestivirus) and reproductive organs (*T. gondii*, *N. caninum* and SBV), as well as other with a systemic distribution (*A. phagocytophilum*). Annual variations and the effect of the sex and age of the animals and their role as reservoirs were also considered.

Materials and methods

Study area and animals

This study was performed in different areas of Spain, located at the southwestern edge of the roe deer range (Corbet 1978). In this country, roe deer was

restricted to mountain areas, although during the last decades it was colonised to woodlands and peripheral agricultural areas (Tellería & Virgós 1997) increasing the probability of contact with domestic ruminants. Roe deer densities in colonised forests are lower than that in mountains (Tellería & Virgós 1997), with values ranging between 1 roe deer/km² in bordering areas to 15–20 roe deer/km² in some hunting farms (Junta de Andalucía 2007).

To perform this study, faecal, blood, myocardium and spleen samples were collected from 93 roe deer hunted from January 2013 to April 2015 in different climatic areas of Spain: Oceanic ($n=44$), Mediterranean ($n=13$), Continental ($n=17$) and Mountainous ($n=18$) determining four different ecosystems (Figure 1), whose characteristics are summarised in Table 1.

For each animal, sex (43 females and 48 males) and age were registered according to their teeth features (Høye 2006). According to their teeth features, the animals were divided in two age-groups, young (<2 years; $n=25$) and adult (>2 years; $n=67$).

Sample collection and laboratorial procedures

All samples were taken during field necropsies, kept under refrigeration and transported to the laboratory as soon as possible. Blood was drawn from the heart or the thoracic cavity; serum was obtained after centrifugation and stored at -20°C . Faeces were taken from the rectum and analysed within 24 h; heart and spleen were stored at -20°C until processed.

Serum samples were analysed using different ELISA tests: Anti-*T. gondii* antibodies were tested with the indirect ELISA LSIVET Ruminant serum Toxoplasmosis (LSI, Lissieu, France). The presence of antibodies against *N. caninum* was analysed using the indirect ELISA, ID Screen *N. caninum* Indirect ELISA (IDVET, Montpellier, France). For Schmallenberg detection, a competitive ELISA (Ingezim Schmallenberg, Ingenasa, Madrid, Spain) was performed. It was also performed for the detection of Pestivirus (LSIVet TM Ruminant BVD/BD p80-Serum) and Herpesvirus (LSIVet TM Bovine IBR gB-Serum). All the commercial tests were used according to the manufacturer's instructions; absorbance values were measured using a spectrophotometer (680XR; Bio-Rad laboratories). Anti-*C. stimulator* antibodies were detected by an in-house indirect ELISA with Excretory/Secretory (ES) antigens described by Arias et al. (2014).

For *A. phagocytophilum* and *C. burnetii* detection, total DNA was extracted from spleen samples using a DNA purification kit for tissues (Nucleospin Tissue DNA

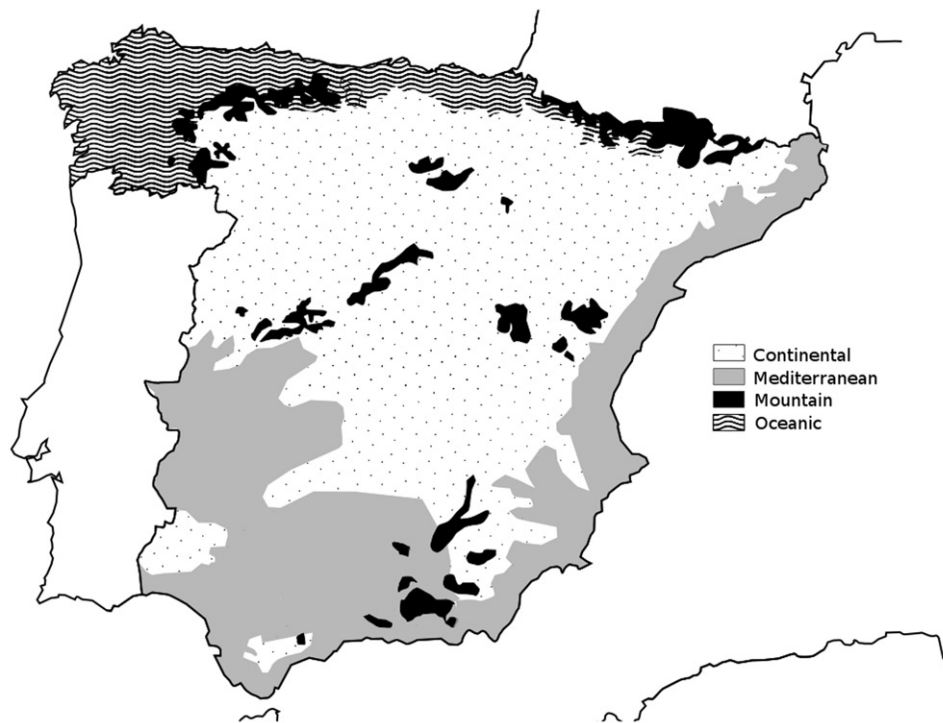


Figure 1. Map of Spain representing the different climatic areas.

Table 1. Characteristics of the climatic areas of origin of roe deer.

Climate	Sampled animals	Mean altitude, m	Annual mean temperature, °C	Annual mean precipitations, mm	Vegetation
Oceanic	44	630	12 – 14	>1000	Deciduous atlantic forests with <i>Fagus sylvatica</i> , <i>Quercus robur</i> and <i>Castanea sativa</i>
Continental	17	900 – 1100	10 – 11	500–550	Not forming forest trees, <i>Quercus ilex</i> , <i>Pinus</i> and <i>Quercus faginea</i>
Mediterranean	13	185	17	800	Evergreen trees, <i>Quercus ilex</i> and <i>Quercus suber</i>
Mountainous	18	>1200	5	>1000	Scarce vegetation, <i>Pinus</i> and <i>Quercus</i>

extraction kit, Macherey-Nagel, Düren, Germany), while for MAP detection total DNA was purified directly from faeces using a specific stool DNA extraction kit (DNAExtract-VK, Vacunek, Derio, Spain). Subsequently, three commercial qPCR kits were performed according to the manufacturers' instructions, based on the targets *msp4* (*A. phagocytophilum* Advanced Kit, Genesig, Southampton, UK), *IS1111* (TaqVet *Coxiella burnetii*, LSI, Lissieu, France) and F57 (ParaTB Kuant-VK, Vacunek, Derio, Spain) for *A. phagocytophilum*, *C. burnetii* and MAP, respectively. All qPCR assays were analysed in a thermocycler Applied Biosystems® 7500 (Life Technologies, Carlsbad, CA).

Digestive parasites (*Eimeria*, *Moniezia* and GI nematodes) were detected in faeces by saline flotation (MAFF 1986). Lungworms (*D. noerteri* and *V. capreoli*) were detected by the Baermann technique (Baermann 1917; Wetzel 1930). Finally, *Sarcocystis* was detected in the myocardium by the observation of microcysts by

the compression method described by Pérez-Creo et al. (2013).

Statistical analysis

In order to analyse the influence of some factors on infections, variables were grouped and categorised for statistical analysis as Age: 1 (≤ 2 years), 2 (> 2 years); Climatic area: 1 (Oceanic), 2 (Mountainous), 3 (Mediterranean) and 4 (Continental); Sex: 0 (female), 1 (male); Sampling year: 2013, 2014 and 2015.

The risk of being infected by every pathogen was evaluated by a logistic regression analysis using the `glm()` function in R statistical package. The dependent variable was a positive (0–1) response to the pathogen. Factors indicated previously and all of the other infections were introduced in a backward conditional method and removed from the model one by one by the `stepAIC()` function on the basis of the lowest AIC

Table 2. Prevalence of infectious and parasitic agents in roe deer from Spain.

Agent (location)	Prevalence, %	95%confidence interval
Cardio-respiratory		
<i>Sarcocystis</i> spp.	88.8 (79/89)	79.9 – 94.2
<i>V. capreoli</i>	38.0 (35/92)	28.1 – 48.7
<i>D. noerteri</i>	2.4 (6/92)	2.4 – 13.6
<i>C. stimulator</i>	23.8 (20/84)	15.2 – 34.3
Digestive		
Gastrointestinal nematodes	62.9 (56/89)	52.0 – 72.9
<i>Eimeria</i> spp.	29.2 (26/89)	20.0 – 39.8
<i>Moniezia</i> spp.	6.7 (6/89)	2.5 – 14.0
<i>M. avium</i> subs. <i>paratuberculosis</i>	1.5 (1/66)	0.04 – 8.1
Pestivirus	0.0 (0/84)	0.0 – 0.06
Bovine Herpesvirus	0.0 (0/84)	0.0 – 0.06
Reproductive		
<i>Toxoplasma</i>	25 (21/84)	16.2 – 35.6
SBV	53.6 (45/84)	42.3 – 64.5
<i>N. caninum</i>	0.0 (0/84)	0.0 – 0.06
Systemic		
<i>A. phagocytophilum</i>	34.2 (26/76)	23.7 – 46.0

value until the best model was built. Odds ratio was computed by raising e to the power of the logistic coefficient over the first category of the factor. All statistical analyses were done in the R statistical package (R v.3.1.1; R Development Core Team (R Core Team R 2015)).

Results

The results obtained in this study for the different agents are summarised in Table 2. At least one pathogen was found in 95.7% of roe deer and a high percentage (91.3%) harboured more than two pathogens. *Sarcocystis* was the most frequently diagnosed agent, followed by GI nematodes and SBV. *Varestrongylus capreoli*, *A. phagocytophilum*, *Eimeria*, *T. gondii* and *C. stimulator* displayed medium prevalences and low percentages were registered for *Moniezia*, *D. noerteri* and MAP. No infections by *N. caninum*, Bovine herpesvirus, pestivirus or *C. burnetii* were found (Table 2).

Table 3 shows the results obtained in the logistic regression model. Climate was significantly associated with the prevalence of *T. gondii*, *A. phagocytophilum* and *C. stimulator*. Roe deer from Oceanic ecosystems have a higher risk to be infected by *T. gondii* than those from continental areas. Similarly, they are 7.8 and 42.7 times more likely to be infected by *A. phagocytophilum* in comparison with those from Continental and Mountainous areas, respectively, and finally, they have also 13.3 times more probabilities of being infested by *C. stimulator* than those from Mediterranean areas.

Varestrongylus capreoli was absent in the Mediterranean area and *D. noerteri* in Mediterranean and Continental populations. MAP DNA was detected

only in one of the four populations of roe deer analysed (Oceanic) and *Moniezia* was detected in Oceanic and Mountainous areas (Data not shown).

Moreover, *C. stimulator* and *A. phagocytophilum* displayed annual variations with higher prevalences in 2013 ($p < .05$). Only sex was related to the prevalence of SBV; females have 2.94 times more risk to be infected by SBV than males. Finally, age was not classified as a risk factor for any of the analysed agents.

Respect the relations between the different agents; a positive relation was detected between *Eimeria* spp. and GI nematodes and between *V. capreoli* and SBV. In contrast, a negative relation was observed between *V. capreoli* and *C. stimulator*.

Discussion

This study represents the first large-scale survey including infectious and parasitic agents affecting Spanish roe deer, giving an overview on the integral sanitary status of this ungulate. A very high percentage of the animals included in this study resulted positive for at least one pathogen, and most of them were in contact with more than two agents, indicating widespread pathogen circulation in roe deer populations from the Iberian Peninsula. Those results are in contrast to the results of Boadella et al. (2010) who hypothesised that roe deer would not be an important species for wildlife disease surveillance as its non-gregarious behaviour would lead to fewer intra-specific contacts and its selective feeding behaviour could lead to less inter-specific contacts.

Expansion of roe deer over the last decades may have had contradictory effects on ecosystems (Valente et al. 2014), including the promotion of disease transmission through both increased intra- and inter-specific contact rates, with an influence in the epidemiology of several contagious diseases potentially shared with other native wild and domestic ungulates, and even human beings (Gortazar et al. 2007).

Our results showed that *Sarcocystis*, followed by GI nematodes and SBVs are the most prevalent agents in roe deer from Spain. Those agents are very prevalent and widespread by the whole territory, without significant differences between the four climatic areas and without variations overtime. The high level of infection by *Sarcocystis* reveals the efficacy of this prey–predator life-cycle in Spain that could be the consequence of high ungulate densities and the presence of large carnivores–predators that quickly eliminate animals weakened by the disease (Kusak et al. 2012; Pérez-Creo et al. 2013). In our opinion, the predation effect due to

Table 3. Last step of a logistic regression.

Factor	Estimate	S.E.	<i>p</i>	OR	Lower ^a	Upper ^a
<i>T. gondii</i> infection						
Intercept	-0.4238	0.4800	.377	0.65	0.255	1.677
Climate 1	-	-	-	-	-	-
Climate 2	-0.4983	0.8016	.534	0.61	0.126	2.923
Climate 3	-0.1303	0.7233	.857	0.88	0.213	3.623
Climate 4	-2.1952	1.1336	.053	0.11	0.012	1.027
<i>C. stimulator</i> infection						
Intercept	2.0027	0.8408	.017*	7.41	1.426	38.500
2013	-	-	-	-	-	-
2014	-3.0070	0.8150	.001***	0.05	0.010	0.244
2015	-2.6839	1.1073	.015*	0.07	0.008	0.598
Climate 1	-	-	-	-	-	-
Climate 2	-1.3479	1.1246	.231	0.26	0.029	2.354
Climate 3	-2.5902	1.1356	.023*	0.07	0.008	0.695
Climate 4	-1.3299	0.9274	.152	0.26	0.043	1.629
<i>Vaestrongylus</i>	-1.7342	0.7718	.025*	0.18	0.039	0.801
Schmallenberg infection						
Intercept	0.7732	0.3490	.0267*	2.16	1.093	4.294
Sex	-1.0868	0.4614	.0185*	0.34	0.136	0.833
<i>A. phagocytophilum</i> infection						
Intercept	1.7010	0.7267	.019*	5.48	1.319	22.769
2013	-	-	-	-	-	-
2014	-1.7145	0.6939	.014*	0.18	0.046	0.702
2015	-18.2671	1385.3780	.990	1.17 10 ⁻⁰⁸	0.000	Inf
Climate 1	-	-	-	-	-	-
Climate 2	-2.0583	0.8089	.011*	0.13	0.026154646	0.623
Climate 3	-1.0837	0.7891	.170	0.34	0.072062190	1.589
Climate 4	-3.7535	1.2130	.002**	0.02	0.002	0.253
Gastrointestinal nematode infection						
Intercept	0.1591	0.2528	.529	1.17	0.714	1.924
<i>Eimeria</i>	1.5457	0.5994	.010**	4.69	1.449	15.189
<i>Eimeria</i> infection						
Intercept	-1.9810	0.5334	.001***	0.14	0.048	0.392
GI nematodes	1.5457	0.5995	.010**	4.69	1.449	15.190
<i>V. capreoli</i> infection						
Intercept	-1.0756	0.4416	.015*	0.34	0.143	0.810
Schmallenberg	1.5402	0.5746	.007**	4.66	1.513	14.389

Factors have been eliminated step by step until the best model.

^a95%CI for OR.

p* < .05; *p* < .01; ****p* < .001.

a parallel increase of large predators as wolves that have increased their populations (natural re-colonization or introduction into systems where they have been absent for a long period) could have had a detrimental impact on roe deer populations, as revealed in the last 5 years in northwestern Spain. However, it should be considered that predation could represent the ultimate cause of death in ungulates with other factors, such as diseases or starvation, which are the original cause of the death (Andrén & Liberg 2015).

Gastrointestinal nematodes were also very frequent in roe deer from Spain. The logistic regression model showed that roe deer infected with GI nematodes have significantly higher risk (OR 4.69) to be infected with *Eimeria*; both parasites have a direct life cycle determined by the survival of the infecting stages in the environment. Boadella et al. (2010) suggested that seroprevalences in roe deer are largely determined by environmental factors, potentially modulating vector

populations or pathogen survival in the environment. Previous studies in NW Spain have revealed that roe deer may act as a potential reservoir for domestic ruminants for some species of gastrointestinal nematodes (Pato et al. 2013) but not for *Eimeria* due to the lack of cross infections (Díaz et al. 2010).

Viral and bacterial agents displayed very low prevalences in this study, but for SBV and *A. phagocytophilum*, both transmitted by vectors. Those results are close to that found in previous studies in Spain by Boadella et al. (2010) who explained these low percentages by the characteristic behaviour of this ungulate that would lead to fewer intra- and inter-specific contacts. However, this explanation is in contradiction with the high prevalence registered in this study for some direct life-cycle parasites, such as GI nematodes and *Eimeria*. The absence of herpesvirus and pestivirus in this study coincides with the low prevalence detected by Boadella et al. (2010) in previous studies

in Spain or by Candela et al. (2014) in France and reflects a relative isolation of roe deer from domestic animals.

Only one adult female from the Oceanic area was infected by *M. avium* subsp. *paratuberculosis*; those results confirm the low prevalence of this agent, even in areas with high probability of contact with domestic ruminants. Most investigations carried out in wild ruminants in Spain showed low prevalences between 0–2.9% (Aguirre et al. 1999; Gutiérrez 2000). However, in a recent study Boadella et al. (2010) in different populations from Spain detected a MAP seroprevalence of 9.2%, but they tested negative for PCR, suggesting the limited role of this ungulate in the epidemiology of this disease.

The absence of *C. burnetti* in our study contrast with the 15% found by Ruiz-Fons et al. (2008), and indicates that the circulation of these bacteria, transmitted by contaminated aerosols and by ticks, in roe deer populations is low, suggesting that they did not constitute an important reservoir for both domestic animals and humans.

According to our results, except for *C. burnetti*, vector-borne diseases have medium to high seroprevalences in roe deer populations. The prevalence for *A. phagocytophilum* was 34.2%. It is worth noting that the prevalence of *A. phagocytophilum* in roe deer from Europe presents high variability depending on the country; while only 5.1% of roe deer were positive in Italy (Carpi et al. 2009), the prevalence in the Bavarian region (Germany), where ticks and roe deer are in close contact, raised to 98.9% (Overzier et al. 2013). In Spain, only two studies have been performed in roe deer up-to-now. Our results are similar to those from Oporto et al. (2003), who reported a prevalence of 38% in the North of the country. In contrast, De la Fuente et al. (2008) established a prevalence of 18% in the South, as well as the presence of other species such as *A. ovis* and *A. marginale* in roe deer. The prevalence of *A. phagocytophilum* was higher in the Oceanic than in the Mountainous and Continental areas of the Peninsula. This fact may be associated with climatological conditions, especially the high humidity, which is favourable to the survival of *Ixodes ricinus* ticks, which is the most prevalent species in the roe deer from Oceanic climate (Vázquez et al. 2011). Annual variations were detected in the prevalence of *A. phagocytophilum*, with higher percentages in 2013 in relation to 2014.

The seroprevalence values for SBV, transmitted through culicoides biting midges, were similar to that found (45.9%) in Belgium by Linden et al. (2012). In Spain, only two studies have detected SBV positive roe

deer (Fernández-Aguilar et al. 2014; Díaz et al. 2015), and the results indicated rapid spread among roe deer since the emergence of virus. In this regard, our study adds new information, showing that the virus is present in the whole country, with no significant differences between the areas of study. Moreover, differences were detected with respect to the sex of the animals, with females having more seropositivity than males; this fact can have clinical relevance as Schmallenberg infections in cows can cause reproductive disorders.

None of the studied animals presented antibodies against *N. caninum*. On the contrary, a high and widespread seroprevalence against *T. gondii* was observed in three of the populations studied. The oocysts of *T. gondii* are excreted in the environment by cats that act as definitive hosts. Those oocysts have high environmental resistance in favourable temperate and moist conditions. In addition, our results confirm the high relevance of environmental conditions in the risk of exposure to *T. gondii*. Neither age nor sex was associated with seropositivity. This study indicates that roe deer is involved in the life-cycle of *T. gondii* and according to Vikoren et al. (2004) and Panadero et al. (2010) who have suggested that among cervids, roe deer is the most susceptible to *T. gondii* and is a potential source of infection for other domestic ruminants and humans. Our results agree with those previously reported (Gauss et al. 2006; Gamarra et al. 2008) in cervids from different areas of Spain (21.8–33.9%). Significant differences between areas were found; animals from the Continental area, with scarce precipitations (Table 1), presented a significant lower seroprevalence than roe deer hunted in oceanic areas. Our results are consistent with previous reports showing a positive correlation between the seropositivity and the mean annual rainfall (Gamarra et al. 2008); factors such as humidity and moderate temperatures may play an important role in the survival and sporulation of *T. gondii* oocysts, and consequently in the probability of acquiring the infection (Smith & Frenkel 1995).

In relation to the absence of *N. caninum*, in roe deer from Spain, seroprevalence by *N. caninum* (6.1%) in previous studies was low (Panadero et al. 2010); nevertheless, the seropositivity rates were higher for other species, including wild ruminants (Almería et al. 2007). These results suggest that the transmission of the disease is not effective considering the living conditions of the roe deer from Spain. Compared to *T. gondii*, definitive hosts are supposed to shed a lower number of *N. caninum* oocysts and the range of intermediate hosts is less wide (Dubey & Schares 2011; Candela et al. 2014). Consequently, the horizontal transmission of this protozoan might be more limited.

The participation of wildlife on the *N. caninum* life-cycle remains unknown, but it seems that roe deer do not play an important role, in contrast to other wild ruminant species such as red deer (Almería et al. 2007).

In relation to the lungworms, *V. capreoli* was much more abundant than *D. noerteri*. This observation was similar to that observed by Panadero et al. (2001) in roe deer from northwestern Spain, but the percentages were higher than in our study (62% and 18.2%, respectively). Considering that the species recorded in this study are highly host specific it is unlikely that roe deer represent a significant reservoir of lungworms transmissible to domestic ruminants.

Finally, it should be pointed out the high seroprevalence of throat botfly *C. stimulator* found in all climatic areas, showing that this myiasis since their detection in 2001 by Notario and Castresana (2001) in an animal imported from France, has spread for almost all the Peninsula (Calero-Bernal & Habela 2013; Arias et al. 2014) although the prevalence was significantly higher in areas with oceanic climate in relation to those with Mediterranean climate; moreover, the seroprevalence in 2013 was higher than in the following 2 years. The prevalence of this myiasis seems to be very influenced by weather conditions. Climate directly influences the development of the free stages of the parasite, pupae and adult flies, affecting the chronology and the intensity of infestation (Calero-Bernal & Habela 2013).

Conclusions

In conclusion, the high prevalence registered for some agents, affecting the roe deer cardiorespiratory system, especially *Sarcocystis* and the throat botfly, may have significant consequences for their health status making them an easy prey for large carnivores. Moreover, a high percentage of roe deer were infected by digestive parasites, especially by GI nematodes and *Eimeria*, which could provoke alterations in food digestion and absorption, resulting in debilitation. Furthermore, although not demonstrated in roe deer, reproductive diseases provoked by *T. gondii* and SBVs may lead to a decrease in female fecundity and early juvenile survival. Overall, infections, especially those of parasitic aetiology, affecting Spanish roe deer can represent one of the causes of the descent in the abundance of this ungulate registered in the last years, and the high prevalence of zoonotic agents such as *T. gondii* and *A. phagocytophilum* could also have a great relevance in the environmental and/or Public Health.

Acknowledgements

We thank the Spanish Roe deer Association (ACE), and particularly gamekeepers and hunters for their inestimable collaboration in the collection of samples.

Disclosure statement

The authors report no conflicts of interest.

Funding

This research was supported by a Programme for consolidating and structuring competitive research groups (GRC2015/003, Xunta de Galicia) and by the Research Project 'RUMIGAL: Rede de estudo multidisciplinar dos ruminantes en Galicia' (R2014/005, Xunta de Galicia, Spain).

References

- Aguirre A, Bröjer C, Mörner T. 1999. Descriptive epidemiology of roe deer mortality in Sweden. *J Wildl Dis.* 35:753–762.
- Almería S, Vidal D, Ferrer D, Pbon M, Fernandez-De-Mera MIG, Ruiz-Fons F, Alzaga V, Marco I, Calvete C, Lavin S. 2007. Seroprevalence of *Neospora caninum* in non-carnivorous wildlife from Spain. *Vet Parasitol.* 143:21–28.
- Andrén H, Liberg O. 2015. Large impact of Eurasian lynx predation on roe deer population dynamics. *PLoS One.* 10: e0120570.
- Arias MS, Pajares G, Paz-Silva A, Díez-Baños N, Suárez JL, Díez-Baños P, Sánchez-Andrade R, Morrondo P. 2014. Antigen characterization from second instars of oestrid bot flies for the detection of anti-*Cephenemyia stimulator* antibodies by ELISA in roe deer (*Capreolus capreolus*). *Med Vet Entomol.* 28:83–89.
- Baermann G. 1917. Eine einfache Methode sur Auffindung von Ankylostomum-(Nematoden)-larven in Erdproben. *Gneesk Tijdschr Nederl-Indië.* 57:131–137.
- Boadella M, Carta T, Oleaga A, Pajares G, Muñoz M, Gortázar C. 2010. Serosurvey for selected pathogens in Iberian roe deer. *BMC Vet Res.* 6:51. doi: 10.1186/1746-6148-6-51.
- Calero-Bernal R, Habela MA. 2013. First report of *Cephenemyia stimulator* (Diptera, Oestridae) parasitizing Roe deer (*Capreolus capreolus*) in Extremadura (Spain). *Galemys.* 25:29–34.
- Candela MG, Serrano E, Sevilla J, León L, Caro MR, Verheyden H. 2014. Pathogens of zoonotic and biological importance in roe deer (*Capreolus capreolus*): seroprevalence in an agro-system population in France. *Res Vet Sci.* 96:254–259.
- Carpi G, Bertolitti L, Pecchioli E, Cagnacci F, Rizzoli A. 2009. *Anaplasma phagocytophilum* groEL gene heterogeneity in *Ixodes ricinus* larvae feeding on roe deer in Northwestern Italy. *Vector Borne Zoonotic Dis.* 2:179–184.
- Carranza J. 2010. Ungulates and their management in Spain. In: Apollonio M, Andersen R, Putman R, editors. *European Ungulates and their management in the 21st century.* Cambridge (UK): Cambridge University Press; p. 421–422.
- Corbet GB. 1978. The mammal of the Palearctic Region: a taxonomic review. British Museum (Natural History). London and Ithaca: Cornell University Press; p. 198–204.

- Corner LA. 2006. The role of wild animal populations in the epidemiology of tuberculosis in domestic animals: how to assess the risk. *Vet Microbiol.* 112:303–312.
- Díaz P, Paineira A, Dacal V, Vázquez L, Cienfuegos S, Arias MS, Pato FJ, Paz-Silva A, Panadero R, Sánchez-Andrade R, et al. 2010. *Eimeria* infections in wild (*Capreolus capreolus*) and extensive-reared domestic ruminants from Galicia (NW Spain). *Rev Ibero-Latinoam Parasitol.* 69:83–89.
- Díaz JM, Prieto A, López C, Díaz P, Pérez A, Panadero R, Pajares G, Díez-Baños P, Morrondo P, Fernández G. 2015. High spread of Schmallenberg virus among roe deer (*Capreolus capreolus*) in Spain. *Res Vet Sci.* 102:231–233.
- De La Fuente J, Ruiz-Fons F, Naranjo V, Torina A, Rodríguez O, Gortázar C. 2008. Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. *Res Vet Sci.* 3:382–386.
- Dubey JP. 1994. Toxoplasmosis. *J Am Vet Med Assoc.* 205:1593–1598.
- Dubey JP. 1999. Recent advances in *Neospora* and *Neosporosis*. *Vet Parasitol.* 84:349–367.
- Dubey JP, Schares G. 2011. Neosporosis in animals—the last five years. *Vet Parasitol.* 18:90–108.
- Fandos P, Burón D. 2013. Corzos [Roe deer]. Ed Cervál, Barcelona; 288 p.
- Fernández-Aguilar X, Pujols J, Velarde R, Rosell R, López-Olvera JR, Marco I, Pumarola M, Segalés J, Lavín S, Cabezón O. 2014. Schmallenberg virus circulation in high mountain ecosystem, Spain. *Emerging Infect Dis.* 20:1062–1064.
- Gamarra JA, Cabezón O, Pabón M, Arnal MC, Luco DF, Dubey JP, Dubey JP, Cortázar C, Almería S. 2008. Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain. *Vet Parasitol.* 153:152–156.
- Gauss CBL, Dubey JP, Vidal D, Cabezón O, Ruiz-Fons F, Vicente J, Marco I, Lavín S, Cortázar C, Almería S. 2006. Prevalence of *Toxoplasma gondii* antibodies in red deer (*Cervus elaphus*) and other wild ruminants from Spain. *Vet Parasitol.* 136:193–200.
- Gortázar C, Ferroglio E, Höfle U, Frölich K, Vicente J. 2007. Diseases shared between wildlife and livestock: a European perspective. *Eur J Wildlife Res.* 53:241–256.
- Gutiérrez A. 2000. Estado sanitario de los rumiantes silvestres de las reservas Nacionales de León [Health status of wild ruminants in Leon national preserves] [dissertation]. Facultad de Veterinaria. Universidad de León.
- Høye TT. 2006. Age determination in roe deer: a new approach to tooth wear evaluated on known age in individuals. *Acta Theriol.* 51:205–214.
- Junta de Andalucía. 2007. Manual de conservación y gestión del corzo andaluz. Manuales de conservación de la naturaleza. Consejería de medio ambiente, 4:84.
- Kusak J, Spicic S, Slijepcevic V, Bosnic S, Janje RR, Duvnjak S, Sindjic M, Majnaric D, Cvetnic Z, Huber D. 2012. Health status of red deer and roe deer in Gorski kotar, Croatia. *Vet Arh.* 82:59–73.
- Linden A, Desmecht D, Volpe R, Wirtgen M, Grefoire F, Pirson J, Paternostre J, Kleijnen D, Schirmer H, Beer M, et al. 2012. Epizootic spread of Schmallenberg virus among wild cervids, Belgium, Fall 2011. *Emerging Infect Dis.* 18:2006–2008.
- Morrondo P, Vázquez L, Díez-Baños P. 2010. Parasitic infections of wild ruminants in Spain with special attention to roe deer and chamois. *Parassitologia.* 52:155–158.
- MAFF. 1986. Manual of veterinary parasitological laboratory techniques, (Ministry of Agriculture, Fisheries and Food, Technical Bulletin No. 18. London: HMSO; p. 2–67.
- Notario A, Castresana L. 2001. Contribution to the knowledge of *Cephenemyia stimulator* Clark, 1815 (Diptera, Oestridae) in Spain. *Folia Venatoria.* 30–31:325–326.
- Oporto B, Gil H, Barral M, Hurtado A, Juste RA, García-Pérez AL. 2003. A survey on *Anaplasma phagocytophila* in wild small mammals and roe deer (*Capreolus capreolus*) in Northern Spain. *Ann NY Acad Sci.* 990:98–102.
- Overzier E, Pfister K, Herb I, Mahling M, Bock GJ, Silaghi C. 2013. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks infesting roe deer in Southern Germany. *Ticks Tick Borne Dis.* 4:320–328.
- Panadero R, Carrillo EB, López C, Díez-Baños N, Díez-Baños P, Morrondo MP. 2001. Bronchopulmonary helminths of roe deer (*Capreolus capreolus*) in the northwest of Spain. *Vet Parasitol.* 99:221–229.
- Panadero R, Paineira A, López C, Vázquez L, Paz-Silva A, Díaz P, Dacal V, Cienfuegos S, Fernández G, Lago N, et al. 2010. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). *Res Vet Sci.* 88:111–115.
- Pato FJ, Vázquez L, Díez-Baños N, López C, Sánchez-Andrade R, Fernández G, Díez-Baños P, Panadero R, Díaz P, Morrondo P. 2013. Gastrointestinal nematode infections in roe deer (*Capreolus capreolus*) from the NW of the Iberian Peninsula: assessment of some risk factors. *Vet Parasitol.* 196:136–142.
- Pérez-Creo A, Panadero R, López C, Díaz P, Vázquez L, Díez-Baños P, Morrondo P. 2013. Prevalence and identity of *Sarcocystis* spp. in roe deer (*Capreolus capreolus*) in Spain: a morphological study. *Res Vet Sci.* 95:1036–1040.
- R Core Team R. 2015. A language and environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org>.
- Royo LJ, Pajares G, Alvarez I, Fernández I, Goyache F. 2007. Genetic variability and differentiation in Spanish roe deer (*Capreolus capreolus*): a phylogeographic reassessment within the European framework. *Mol Phylogenetic Evol.* 42:47–61.
- Ruiz-Fons F, Rodríguez O, Torina A, Naranjo V, Gortázar C, de la Fuente J. 2008. Prevalence of *Coxiella burnetii* infection in wild and farmed ungulates. *Vet Microbiol.* 126:282–286.
- Smith DD, Frenkel JK. 1995. Prevalence of antibodies to *Toxoplasma gondii* in wild mammals of Missouri and East Central Kansas: biologic and ecologic considerations of transmission. *J Wildl Dis.* 31:15–21.
- Stuen S, Granquist EG, Silaghi C. 2013. *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. *Front Cell Infect Microbiol.* 3:31
- Tellería JL, Virgós E. 1997. Distribution of an increasing roe deer population in a fragmented Mediterranean landscape. *Ecography.* 20:247–252.
- Valente AM, Fonseca C, Marques TA, Santos JP, Rodrigues R, Torres RT. 2014. Living on the Edge: roe deer (*Capreolus capreolus*). Density in the margins of its geographical range. *PLoS One.* 9:e88459.
- van den Brom R, Lutikholt SJM, Lievaart-Peterson K, Peperkamp NHMT, Mars MH, van der Poel WHM, Vellema P. 2012. Epizootic of ovine congenital malformations

- associated with Schmallenberg virus infection. Tijdschrift Voor Diergeneeskunde. 137:106–111.
- Vázquez L, Panadero R, Dacal V, Pato FJ, López C, Díaz P, Arias MS, Fernández G, Díez-Baños P, Morrondo P. 2011. Tick infestation (Acari: *Ixodidae*) in roe deer (*Capreolus capreolus*) from northwestern Spain: population dynamics and risk stratification. Exp Appl Acarol. 53:399–409.
- Vikoren T, Tharaldsen J, Fredriksen E, Handeland E. 2004. Prevalence of toxoplasma gondii antibodies in wild red deer, roe deer, moose, and reindeer from Norway. Vet Parasitol. 120:159–169.
- Vilcek S, Nettleton TF. 2006. Pestiviruses in wild animals. Vet Microbiol. 116:1–12.
- Wetzel R. 1930. Zur Diagnose der Lungenwurminvasionen bei Rind und Schaf. Dtsch Tierärztl Wschr. 3:49–50.