



## Using integrated wildlife monitoring to prevent future pandemics through one health approach

P. Barroso <sup>a</sup>, D. Relimpio <sup>a</sup>, J.A. Zearra <sup>a</sup>, J.J. Cerón <sup>b</sup>, P. Palencia <sup>a,c</sup>, B. Cardoso <sup>a,e</sup>, E. Ferreras <sup>a</sup>, M. Escobar <sup>d</sup>, G. Cáceres <sup>f</sup>, J.R. López-Olvera <sup>d,\*</sup>, C. Gortázar <sup>a</sup>

<sup>a</sup> SaBio Instituto de Investigación en Recursos Cinegéticos (IREC) CSIC-UCLM-JCCM, 13071 Ciudad Real, Spain

<sup>b</sup> Interdisciplinary Laboratory of Clinical Pathology, Interlab-UMU, University of Murcia, 30003 Murcia, Spain

<sup>c</sup> Department of Veterinary Sciences, University of Turin, 10095 Turin, Italy

<sup>d</sup> Wildlife Ecology & Health (WE&H) research group and Servei d'Ecopatología de Fauna Salvaje (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

<sup>e</sup> CIBIO/InBio, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Porto, Portugal

<sup>f</sup> Epidemiology Department, Ministry of Agriculture, Fisheries and Food, Madrid, Spain

### ARTICLE INFO

#### Keywords:

Anthropogenic imbalances  
Disease risk  
Host community  
Integrated wildlife monitoring  
One health  
Wildlife health monitoring

### ABSTRACT

In the One Health context, Integrated Wildlife Monitoring (IWM) merges wildlife health monitoring (WHM) and host community monitoring to early detect emerging infections, record changes in disease dynamics, and assess the impact of interventions in complex multi-host and multi-pathogen networks. This study reports the deployment and results obtained from a nationwide IWM pilot test in eleven sites representing the habitat diversity of mainland Spain. In each study site, camera-trap networks and sampling of indicator species for antibody and biomarker analysis were used to generate information. The results allowed identifying differences in biodiversity and host community characteristics among the study sites, with a range of 8 to 19 relevant host species per point. The Eurasian wild boar (*Sus scrofa*) was the most connected and central species of the host communities, becoming a key target indicator species for IWM. A negative relationship between biodiversity and disease risk was detected, with a lower number and prevalence of circulating pathogens in the sites with more species in the community and larger network size. However, this overall trend was modified by specific host-community and environmental factors, such as the relative index of wild boar - red deer interactions or the proximity to urban habitats, suggesting that human-driven imbalances may favour pathogen circulation. The effort of incorporating wildlife population monitoring into the currently applied WHM programs to achieve effective IWM was also evaluated, allowing to identify population monitoring as the most time-consuming component, which should be improved in the future. This first nationwide application of IWM allowed to detect drivers and hotspots for disease transmission risk among wildlife, domestic animals, and humans, as well as identifying key target indicator species for monitoring. Moreover, anthropogenic effects such as artificially high wildlife densities and urbanisation were identified as risk factors for disease prevalence and interspecific transmission.

### 1. Introduction

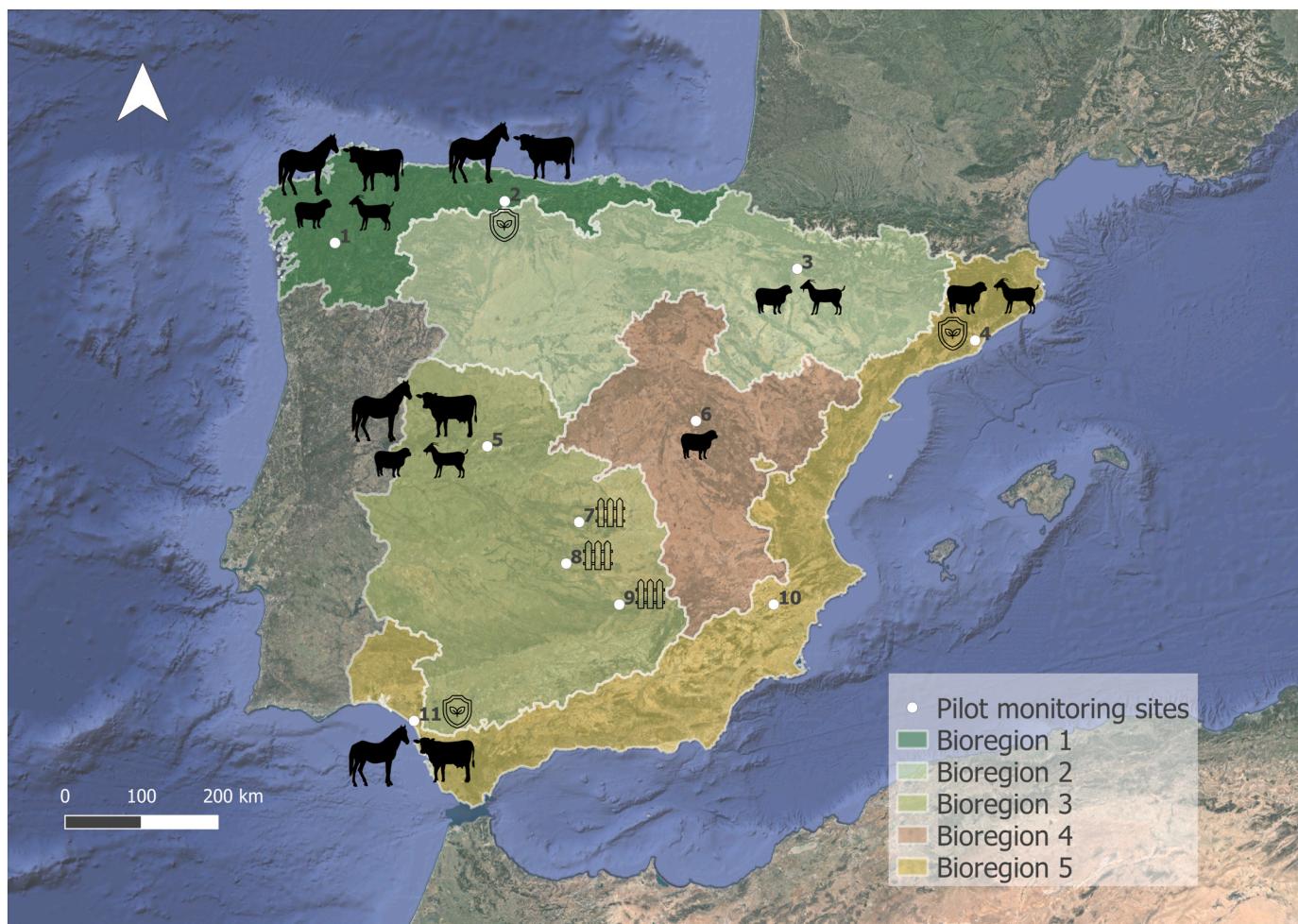
Shared infections at the wild-domestic interface are caused by transmissible pathogens maintained by at least one host species from either compartment [1], and may be relevant to animal health, public health, wildlife management, and biodiversity conservation, with economic, sanitary and ecological impacts [2]. Wildlife is key in the epidemiology of shared diseases [3], and therefore Wildlife Health

Monitoring (WHM) is essential to detect changes in disease occurrence and pathogen emergence, although WHM systems still need substantial improvement [4,5].

While currently most WHM programs rely mainly on general and targeted disease surveillance [6], monitoring wildlife population is required to effectively achieve Integrated Wildlife Monitoring (IWM) from a One Health perspective [4]. The collection of long-term and large-scale information through IWM provides knowledge into the

\* Corresponding author.

E-mail address: [jordi.lopez.olvera@uab.cat](mailto:jordi.lopez.olvera@uab.cat) (J.R. López-Olvera).



**Fig. 1.** Map of the 11 pilot monitoring sites with a division of mainland Spain into five large bioregions (1–5) according to the Spanish Wildlife Disease Surveillance Scheme. Silhouettes of livestock species present at each point are shown. The presence of fences and protected areas are indicated with icons.

epidemiology of pathogens and their establishment as endemic in host populations, which is essential to control shared infections [7]. Thus, a balanced IWM system including wildlife abundance monitoring is key to detect emergent pathogens and changes in pathogen dynamics, to critically assess wildlife disease hazards and the impact of interventions, and to better understand complex multi-host and multi-pathogen networks [8].

The cost and difficulties of covering each disease and host have promoted the search for indicator species [9] and non-specific indicators of population health, including acute-phase proteins (APP), indicators of redox status, and markers of immune system status [10–12].

This study aims to report the implementation of the first nationwide pilot trial of balanced IWM in Europe, using a network of 11 pilot monitoring sites in Spain. The insights obtained in this pilot IWM trial will help to further develop a comprehensive IWM and serve as a reference for implementing IWM systems in other regions.

## 2. Material and methods

### 2.1. Study sites and target species

The study sites selected represented the main habitats, climates and wildlife management systems of mainland Spain [13] (Fig. 1). The Eurasian wild boar (*Sus scrofa*) was selected as the target species due to its abundance, distribution and epidemiological relevance for infections shared between wild and domestic animals and humans [14].

### 2.2. Camera trap survey and calculation of abundance and cross-species interactions

A grid of 20 camera traps (Browning Strike Force HD ProX, Browning Arms Company®, Morgan, Utah, USA) was deployed for two months in each study site following previous studies [15].

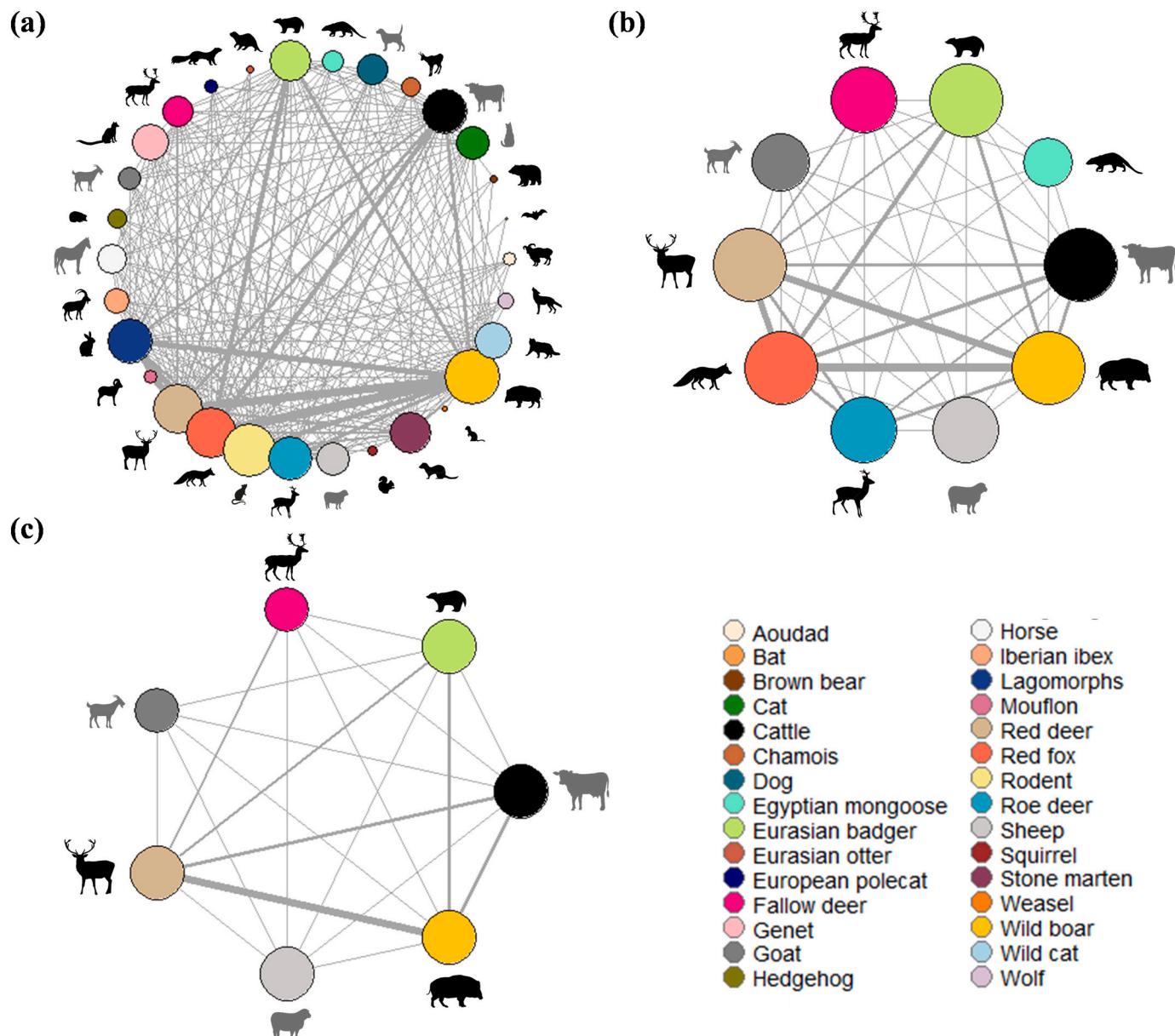
#### 2.2.1. Random Encounter Model method

The Random Encounter Model (REM) has been successfully applied to estimate animal density from camera trap data for different mammal species inhabiting an area, by modelling the rate of random encounters between animals and camera traps without the need for individual recognition [16,17]. Density (individuals/km<sup>2</sup>) was estimated as:

$$D = \frac{y}{t} \frac{\pi}{v \cdot r \cdot (2 + \alpha)}$$

where  $y$  is the number of encounters of the target species,  $t$  is the survey effort,  $v$  is the average daily distance travelled by an individual (day range) [18], and  $r$  and  $\alpha$  are the radius and angle of the camera trap detection zone, respectively. All analyses were carried out using the ‘camtools’, ‘trappingmotion’, and ‘emdbook’ R packages [19,20].

REM was applied to wild boar, red deer (*Cervus elaphus*) and red fox (*Vulpes vulpes*) as the most frequently detected species. Relative abundance indexes, namely trapping rate and relative occupancy index, were calculated for the remaining, less frequently detected species [21] (Table S1).



**Fig. 2.** Social networks of the indirect interactions between (a) all the species, (b) all the potentially implicated hosts in the transmission of the *Mycobacterium tuberculosis complex* (MTC; red deer, roe deer, fallow deer, red fox, wild boar, Egyptian mongoose, Eurasian badger, cattle, sheep and goat), (c) the most epidemiologically relevant hosts for the MTC in Spain (red deer, fallow deer, wild boar, badger, cattle, sheep and goat), obtained by camera trap records in eleven pilot monitoring sites in Spain. Nodes represent different species and edges represent indirect interactions recorded by camera traps between two nodes. The size of the nodes represents the degree and the weight of the edges represents the number of interactions between nodes, evidencing the centrality of the wild boar and the weight of wild boar-red deer interactions. Grey silhouettes: domestic; black: wild. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 2.2.2. Cross-species interactions

Direct interactions were defined as the simultaneous presence of individuals in a photograph. Indirect interactions were defined as the photo-capture of individuals by the same camera trap during a time window of 24 h, based on the environmental survival of the target pathogens [22,23]. The relative direct and indirect intra and inter-specific interaction indexes for the epidemiologically relevant pairs of species were calculated (Table S1).

### 2.3. Social network characterization

Twelve static social networks were constructed, one for each study site and one for all of them together, using the “igraph” R package [24]. Additional networks were constructed (both for each study site and for

the set of 11 study sites) for all the hosts potentially participating in the transmission of the *Mycobacterium tuberculosis complex* (MTC), as well as retaining only the most epidemiologically relevant hosts for MTC in Spain.

The metrics of the nodes and the network were estimated for all the networks using the “igraph” R package (Table S2) [24,25]. The statistical differences in network and node metrics among species and sites were assessed using a Kruskal-Wallis test.

### 2.4. Health monitoring

A total of 398 hunter-harvested wild boar from the 11 study sites (range: 7 to 60, mean  $\pm$  standard error (SE):  $36.2 \pm 0.2$ ) were sampled between 2020 and 2021. Sex and age were recorded, classifying the wild

boars as juveniles (younger than 12 months), yearlings (between 12 and 24 months) or adults (older than two years), according to tooth eruption.

#### 2.4.1. Specific health indicators

Blood samples were collected from the endocranial venous sinus [26]. Sera were obtained by centrifugation at 400g for 5 min and kept at -20 °C until assayed for antibodies against six different pathogens (Table S3).

#### 2.4.2. Non-specific health indicators

Serum concentrations of two positive APPs (Haptoglobin -Hp- and C-reactive protein -CRP-), a negative APP (paraoxonase -Pon1-), two biomarkers of redox balance (serum thiols -Thiol- and cupric reducing antioxidant capacity -Cuprac-), and a marker of immune response (adenosine deaminase -ADA-) were determined as previously described [11,27,28], using an automated analyser (Olympus AU 600, Beckman Coulter). All the assays were validated in the indicator species used in the study, providing inter and intraassay imprecision values lower than 15%, and being linear after serial sample dilutions with saline.

#### 2.5. Land use information

The CORINE land cover categories were compressed into seven broad land-use classes: woodland, scrubland, herbaceous grassland, bare land, arable land, urban land and water habitats [29]. The percentage cover of each land-use class was calculated for each sampling site. Additionally, the straight-line distances in metres from the centroid of each sampling site to the nearest road, urban area, water point, and river were calculated using qGIS 3.4 [30].

#### 2.6. Statistical approach and methods

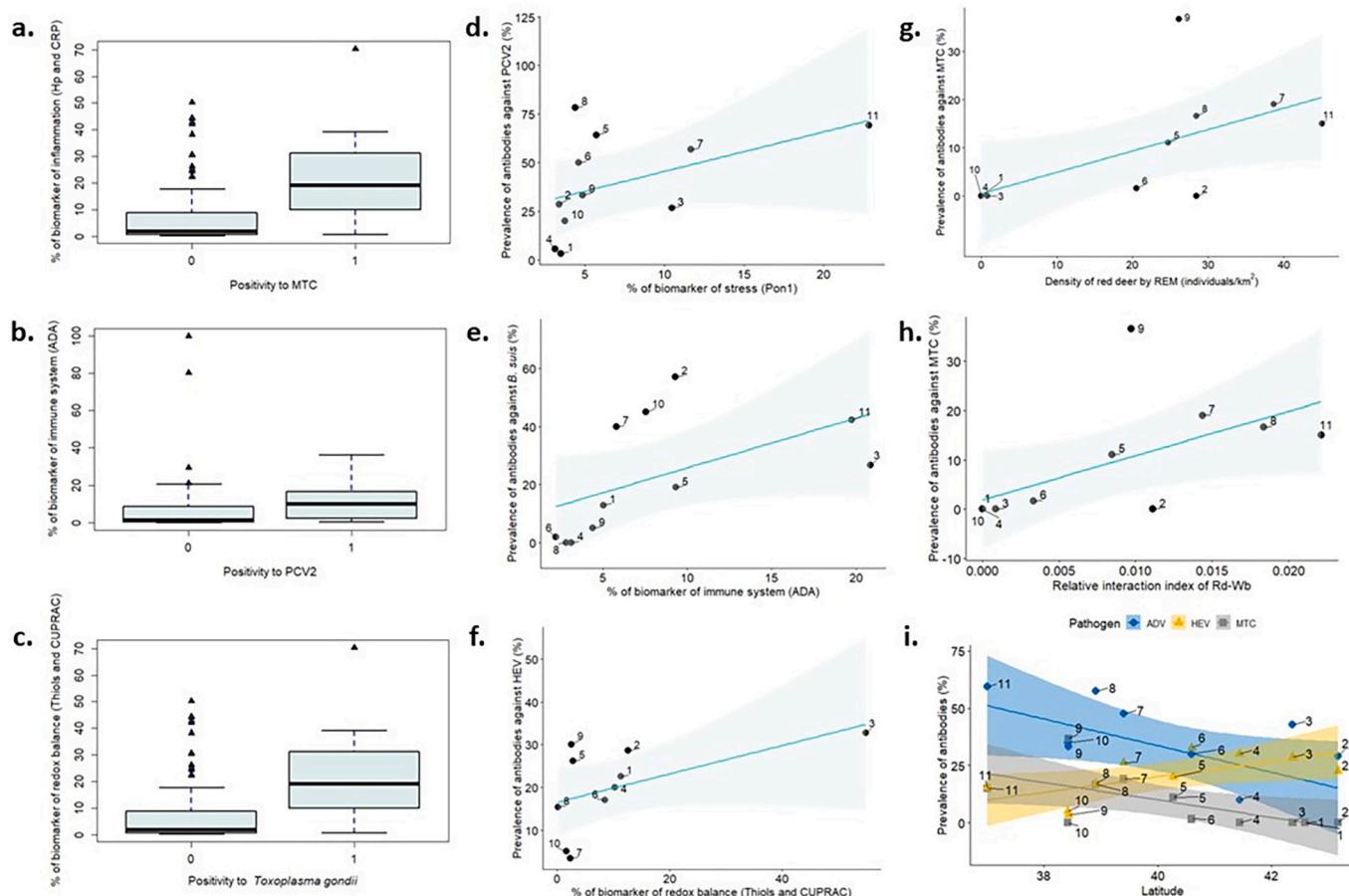
To correct for intersite variability, the concentration and activity values of the unspecific health indicators were transformed to a percentage of the maximum value for each study site (0–100%). The resulting percentages were grouped into four categories, namely biomarker of inflammation (positive APPs: Hp and CRP), biomarker of stress (negative APP: Pon1), biomarker of immune system activation (ADA) and biomarker of redox balance (Cuprac and Thiols). The relationship of the four groups with the presence and prevalence of antibodies against the pathogens studied was evaluated using chi-squared and a Kruskal-Wallis test, respectively. We evaluated spatial differences in these biomarkers through a Kruskal-Wallis test with Dunn's multiple-comparison post hoc analyses [31].

Bivariate analyses were performed to assess the association between populational, environmental and health variables by using Pearson's correlation and Spearman's rank tests according to the distribution of the continuous variables and sample size, and Wilcoxon's sum rank test for the categorical variables (Table S4). The significance of the differences between variables was evaluated using Fisher's test.

Finally, generalized linear mixed models (GLMMs) were fitted to assess the relationship between unspecific biomarkers of health, environmental and population-related variables, and seropositivity to pathogens, selecting as response variables first the biomarker of inflammation (Hp and CRP), and then the seropositivity to MTC (binomial response). As a previous step, collinearity among the explanatory variables was explored [32], and principal component analysis (PCA) was performed to obtain two uncorrelated factors; agricultural and grassland-water habitats (see Table S5). The variables included in the GLMMs are shown in Table S6. The GLMMs were fitted with a gaussian error distribution and the identity link function. The models with an increase in Akaike's Information Criterion lower than two ( $\Delta AIC < 2$ ) were considered suitable to explain the variability observed in the response variable [33]. Once the best model was selected, normality and the absence of residual pattern in data variation were checked. The GLMMs were fitted in the library "lme4" [34]. Significance was set at

**Table 1**  
The average prevalence of antibodies (%  $\pm$  confidence intervals (CI) 95%) against Aujeszky's disease virus (ADV), porcine circovirus type 2 (PCV2), hepatitis E virus (HEV), *Mycobacterium tuberculosis* complex (MTC), *Brucella suis* and *Toxoplasma gondii*, and mean concentration ( $\pm$  standard error) of acute phase proteins (APPs; including C-reactive protein (CRP), haptoglobin (Hp) and paraoxonase 1 (Pon1)), a biomarker of redox balance (serum thiols -Thiol-) and a marker of the immune system (adenosine deaminase -ADA-) in eleven pilot monitoring sites in Spain. Sample size (N) is shown.

Average prevalences (Range)	N	ADV	HPV	PCV2	95% CI	HEV	95% CI	MTC	95% CI	<i>Brucella suis</i>	95% CI	<i>Toxoplasma gondii</i>	95% CI	ADA	Pon1 (IU/mL)	Pon1 (IU/mL)	
Average concentration (Range)	398	32.3 (0.0-58.3)	21.0-43.6 (3.2-78.3)	39.6 (3.2-78.3)	24.4-54.8 (5.0-32.7)	20.1 (0.0-32.7)	17.1-26.0 (0.0-36.7)	9.1 (0.0-36.7)	2.0-16.2 (0.0-57.1)	22.7 (0.0-57.1)	10.5-34.9 (0.0-46.2)	16.3 (0.0-46.2)	6.4-26.2 (0.3-2.2)				
CRP (μg/mL)	398	27.8 ± 8.3 (3.3-71.1)				0.3 ± 0.1 (0.0-0.8)		0.3 ± 0.1 (0.0-1.4)		857.6 ± 856.8 (0.4-9426.0)	84.1 ± 20.4 (22.9-215.9)	0.7 ± 0.2 (0.3-2.2)					



**Fig. 3.** Markers of health status in wild boar from 11 pilot monitoring sites in Spain. Outliers are shown as blue triangles. Percentage of maximum serum concentration (%) of (a) biomarker of inflammation in relation to individual seropositivity to *Mycobacterium tuberculosis* complex (MTC), (b) biomarker of stress in relation to the prevalence of antibodies against porcine circovirus type 2 (PCV2), (c) biomarker of immune system activation in relation to individual seropositivity to PCV2, (d) biomarker of immune system activation in relation to the prevalence of antibodies against *B. suis*, (e) biomarker of redox balance in relation to individual seropositivity to *T. gondii*, and (f) biomarker of redox balance in relation to the prevalence of antibodies against hepatitis E virus (HEV). Integrated wildlife monitoring results on 11 pilot monitoring sites in Spain. Mean prevalence of antibodies against (g) *Mycobacterium tuberculosis* complex (MTC) in relation to the density of red deer (individuals/km<sup>2</sup>) obtained by Random Encounter Model, (h) MTC in relation to the relative interaction index of red deer (Rd)-wild boar (Wb), and (i) Aujeszky's disease virus (ADV; blue), Hepatitis E virus (HEV; yellow) and MTC (grey) in relation to the latitude, at study site level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.05. All analyses were conducted using R software 4.0.2 [35].

### 3. Results

#### 3.1. Population monitoring

The estimated population density values for wild boar (ind/km<sup>2</sup>; mean  $\pm$  SD (range):  $13.39 \pm 0.14$  (0.52–57.49), red deer ( $18.99 \pm 0.19$  (0–41.21), and red fox ( $1.15 \pm 0.01$  (0.31–1.85)) are shown in Table S7. The wild boar was detected in all 11 study sites, red fox in ten sites and red deer in eight (Table S7). All the species detected in each site (mean  $\pm$  SE:  $14 \pm 0.03$ ; range: 8–19) are shown in Table S8.

Table S9 shows the relative interaction index of each pair of species (percentage of camera traps per day) and the percentage of camera traps (%) in which a given interaction was recorded.

The global and study site host networks are shown in Figs. 2 and S1, respectively (doi:<https://doi.org/10.5281/zenodo.6861521>). Fig. S2 displays the networks for all the epidemiologically relevant species implicated in the transmission and maintenance of the MTC in Spain by study site. The network metrics are compiled in Table S10. Fig. S3 shows the node centrality values for each species.

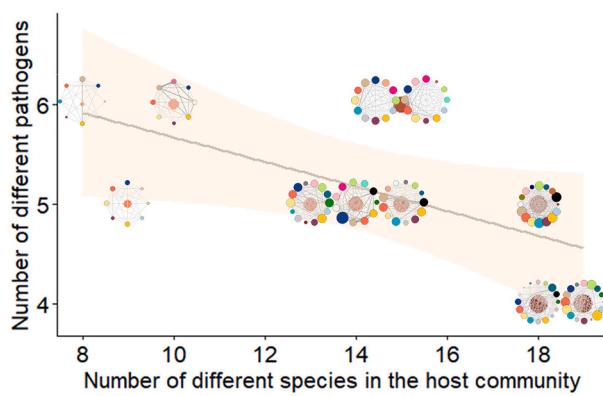
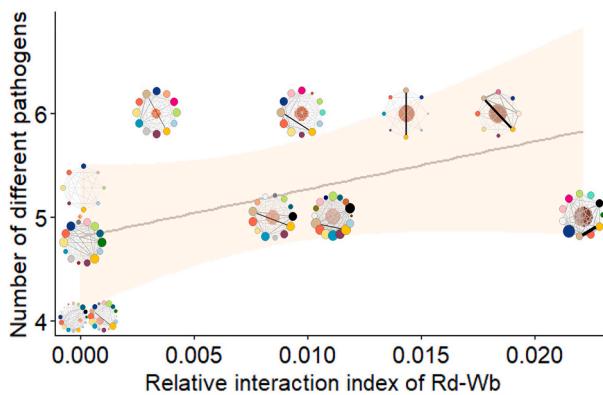
Although red deer occasionally reached higher densities (Table S7),

the wild boar was the key and most connected species of the system, both globally and among the relevant species in MTC epidemiology, as shown by its presence in all study sites (Tables S7 and S8), its connections with other species both overall and among the species relevant in MTC epidemiology (Figs. 2, S1 and S2) and its higher centrality metric values (Fig. S3). Also, the interaction between wild boar and red deer was the most intense one, both overall and when only considering species relevant to MTC epidemiology (Figs. 2, S1 and S2).

#### 3.2. Health monitoring

The prevalences of antibodies against Aujeszky's disease virus (ADV), porcine circovirus type 2 (PCV2), hepatitis E virus (HEV), MTC, *Brucella suis* and *Toxoplasma gondii* detected in wild boar sera are shown in Table S11 and Table 1.

The serum concentration of APPs, Thiols, Cuprac and ADA are shown in Table S12, Table 1 and Fig. S4. The percentage of positive APPs (Hp and CRP, biomarker of inflammation) was significantly higher in wild boar positive to MTC antibodies (W: 175.5,  $p = 0.02$ ). Regarding negative APPs, the activity of Pon1 positively correlated with the prevalence of antibodies against PCV2 (R: 0.62,  $p = 0.03$ ). ADA (biomarker of immune system activation) was positively associated with

**a.****b.**

**Fig. 4.** Integrated wildlife monitoring results on 11 pilot monitoring sites in Spain. Number of different pathogens for which antibodies were found by ELISA in relation to (a) the number of different species detected by camera traps per study site ( $R = -0.61, p \leq 0.05$ ), and (b) the relative interaction index of red deer (Rd) - wild boar (Wb) at study site level ( $R = 0.49, p \leq 0.05$ ). Each dot is surrounded by the local network obtained by camera trapping in such pilot point, with the number of different species recorded (nodes) and the number of interactions (edges). The link between red deer (brown node) and wild boar (yellow node) is highlighted in grey or black, according to the number of interactions, in graph -b-. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PCV2 (W: 423.0,  $p < 0.01$ ) and the prevalence of antibodies against *B. suis* (R: 0.75,  $p < 0.01$ ). Finally, the percentage of the biomarker of the redox balance (Cuprac and Thiols) correlated with the positivity to *T. gondii* (W: 437.0,  $p = 0.05$ ) and the prevalence of antibodies against HEV (R: 0.69,  $p = 0.03$ ) (Fig. 3a-3f).

### 3.3. Integrated monitoring

The correlations between population and environmental variables, and specific (antibody prevalences) and unspecific markers of disease, are shown in Tables S13 and S14, respectively. The prevalence of antibodies against *T. gondii* in wild boar was positively correlated with the trapping rate of free-ranging domestic cats and the percentage of urban habitat (Table S13). The prevalence of antibodies against MTC in wild boar was positively correlated with red deer density and the relative index of wild boar-red deer interactions (Fig. 3g and h). Latitude was negatively correlated with the prevalence of antibodies in wild boar against ADV and MTC and positively for HEV (Fig. 3i). The prevalence of antibodies against ADV in wild boar was positively correlated with the distance to roads or urban areas, and the prevalence against *B. suis* was negatively correlated with the relative interaction index of wild boar-red deer interactions (Table S13). Finally, the number of different pathogens

detected was negatively correlated with the diversity of host species (Fig. 4a) and positively correlated with the relative interaction index of wild boar-red deer interactions (Fig. 4b).

The network size of each study site was negatively correlated with wild boar seroprevalence against MTC ( $R: -0.68, p: 0.02$ ) and ADV ( $R: -0.63, p: 0.04$ ; Fig. S5a). The eigenvector centrality of wild boar was positively correlated with the prevalence of antibodies against PCV2 in this species ( $R: 0.77, p < 0.01$ ; Fig. S5b) and with its population density ( $R: 0.64, p: 0.04$ ; Fig. S5c).

The best GLMM selected (Table S15) explained the seropositivity to MTC (as specific marker of health status) as a function of serum concentration of the biomarker of inflammation (CRP and Hp), wild boar density, and population management in terms of fencing ( $wi = 0.63, R^2 = 31.30\%$ , Table S16a). Wild boar with higher concentrations of CRP and Hp, as well as those from high-density fenced populations had a higher probability of being seropositive to MTC (Table S16a).

The variability in Hp and CRP as unspecific markers of health status was explained by the seropositivity to MTC (specific marker of disease), wild boar density, population management in terms of fencing, and proportion of urban lands ( $wi = 0.66, R^2 = 14.40\%$ , Table S16b). Consequently, wild boar seropositive to MTC had higher concentrations of CRP and Hp and were sampled in dense non-urban fenced populations (Table S16b).

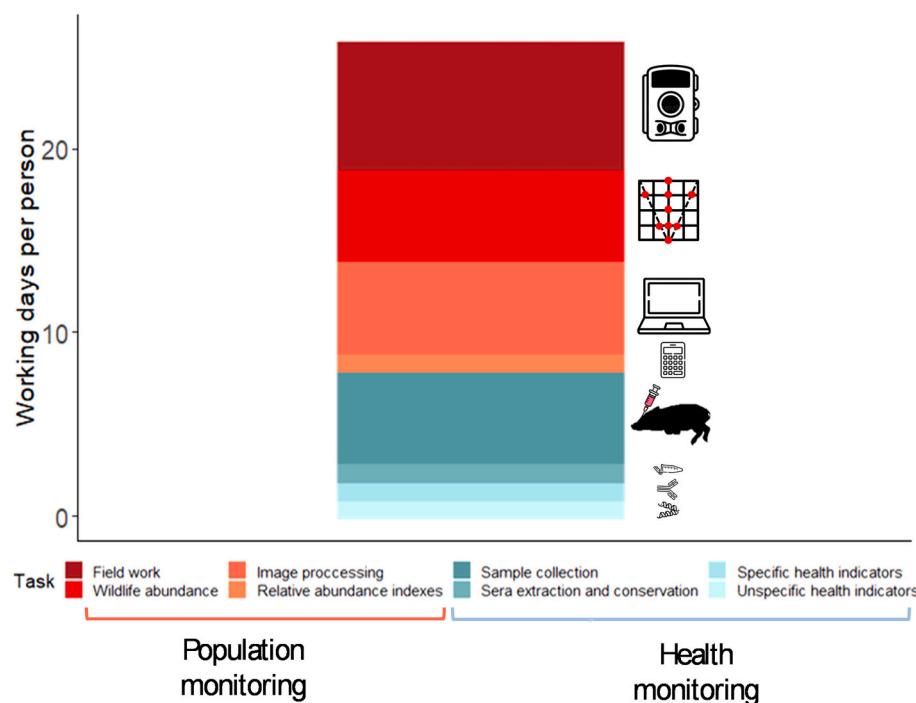
Finally, Fig. 5 shows the estimated distribution of the costs associated to the implementation and development of the IWM experimental pilot trial.

## 4. Discussion

By combining WHM with wildlife population assessment using a One Health approach [4], this pilot IWM yielded novel insights on the effect of host community composition, population abundance and management on the epidemiology of shared infections [1]. This opens the door for the establishment of community-based wildlife health indicators and the identification of hotspots for disease spread and transmission [9].

Higher biodiversity and larger network size reduced the number and prevalence of circulating pathogens (Figs. 4a and S5a) as proposed by the “dilution effect” hypothesis [36] but opposite to previous studies [37]. The “dilution effect” hypothesis postulates that pathogens are less likely to encounter their competent hosts, i.e., individuals in which a given pathogen can replicate and spread to a new susceptible individual, in species-rich communities. Thus, biodiversity would lead to lower host exposure to pathogens and reduced transmission rates [36,38]. However, this overall trend was modulated by specific host-community and environmental factors, such as the density and interaction of wild boar and red deer, two key reservoir species for MTC (Fig. 3g and h) [39,40]. More importantly, the interaction of these two species also increased the overall number of pathogens detected (Fig. 4b), suggesting that population management leading to imbalances may favour pathogen circulation [37]. Similarly, the higher density and spatial aggregation of wild boar and other ungulates in the intensively managed fenced hunting estates of south-western Spain favours disease transmission at the wildlife-livestock interface [22,41], increasing MTC prevalence (Table 16a) and explaining the negative correlation of ADV and MTC seroprevalence with latitude (Fig. 3i) [41–43]. The higher intraspecific wild boar contact rates in these overabundant populations would also explain the negative relationship between wild boar network centrality and the seroprevalence of the pig pathogen PCV2 (Fig. S5b) [44], further supporting the dilution effect of biodiversity [36]. Apart from wildlife management, environmental anthropization - i.e., a set of processes transforming or adapting landscapes by human actions [45]- also favoured pathogen circulation [46,47], as shown by the higher *T. gondii* prevalence in wild boar from periurban habitats with stray cats, agreeing with previous reports [48].

The distribution, abundance and centrality of wild boar (Tables S7 and S8, Figs. S3 and S5c) and its connections with the other species,



**Fig. 5.** Sampling effort of carrying out integrated wildlife monitoring (IWM) in each pilot monitoring site in Spain ( $n = 11$ ). Effort (full working days (8 h) per person) required for population (69.2% of effort; including field work, image processing, calculation of the alternative abundance indicators, and application of Random Encounter Model (REM) method from camera trap data to estimate wildlife abundance) and health monitoring (30.7% of effort; sample collection, sera extraction, and laboratory analyses for specific and unspecific health indicators) per study site.

particularly with those relevant in MTC epidemiology (Table S8, Fig. 2, S1 and S2), make this species key for the introduction, spread and maintenance of diseases in the network [49,57]. Moreover, the wild boar is a spreader and reservoir of pathogens shared between wildlife and livestock [39], presenting high excretion rates and therefore a high risk of infection transmission [40]. Thus, the wild boar is a suitable target species for targeted WHM [4] and to design and implement IWM at the wildlife-livestock interface in the European context.

Overall, the unspecific markers of disease responded to population, environmental and disease factors (Table S14) and responded to the same determinants as the specific disease marker assessed (seropositivity to MTC) (Tables 2, S15a and S15b). Higher antibody prevalences were positively associated with biomarkers of inflammation (MTC and Hp and CRP), stress (PCV2 and Pon 1), activation of the immune system (PCV2 and *B. suis* and ADA), and redox balance (*T. gondii* and Cuprac and Thiols) (Table S14) agreeing with previous reports [11,12,50]. The increase in inflammation biomarkers (Hp and CRP) is often correlated with the extent of lesions [51], and these analytes have lower sensitivity to viral diseases when compared to bacterial infections [52]. Some viruses may even attenuate inflammatory responses to ease infection [53], which could explain the negative relationship of the seroprevalence against HEV and Hp (Table S14). Apart from diseases, the unspecific markers were also affected by environmental variables such as urban cover (Pon 1), cropland (Hp and Pon 1), and the number of different hosts in the network (Cuprac and Thiols) (Table S14). This, together with the great variability among study sites of these unspecific markers (Table S12), shows that considering interactions between environmental, population and epidemiological variables and establishing baseline values for each population is required in order to use them as reliable indicators of health for IWM [10,54]. Although unspecific markers have the potential to become reliable indicators for IWM, their non-specificity is simultaneously their main advantage and drawback, since it can be cheaper and easier to perform than analyzing all the potential pathogens individually but requires defining the conditions influencing these indicators for each population. In general, methods employed to measure markers of redox balance and immune response potentially work for any mammal species, although species-specific

validation is needed. However, the proteins and methods selected could vary for APPs in some groups such as ruminants [55].

This study also allowed quantifying the effort required by each of the components of IWM, namely health and population monitoring, which must be balanced and integrated. The additional efforts to effectively implement IWM depend on the WHM schemes currently applied, which differ significantly among European countries [6]. Due to the traditional approach of WHM as a combination of general and targeted surveillance [6], population monitoring is likely the main required improvement for all WHM schemes [4]. However, this key information is precisely the most costly and time-consuming component of IWM (Fig. 5).

Although the REM methodology used in this pilot study already optimized the labor cost when characterizing the host community [16], further decreasing the effort required to reach the essential reliable population monitoring would increase the feasibility of IWM and facilitate the generalization of its application to wider geographical contexts [15]. Apart from reducing the workload of population monitoring, efforts to improve IWM efficiency by developing, validating, and establishing non-specific markers of disease and/or wildlife population health status deserve further investigation. This would be easier and more cost-effective than targeting several pathogens. Also, non-invasive environmental DNA sampling could help to broaden the spectrum of hosts and pathogens to survey, decreasing sampling effort [56]. Furthermore, planning a central national wild animal serum repository would be desirable to enable researchers and agencies for traceback of pathogens in the future. Finally, the number and diversity of monitoring sites needs to be broadened to reach a comprehensive and representative coverage of peninsular Spain while considering the challenging administrative structure of the country and the different agents involved, as previously recommended [6].

## 5. Conclusion

The implementation of a nationwide pilot IWM scheme showed the added value of combining general and targeted health surveillance with population monitoring and host community characterization. Thereby, IWM steps up from wildlife disease surveillance linking host abundance

and community complexity with health surveillance and disease epidemiology. However, reaching a balance among the three pillars of IWM and designing a scheme that is technically sound, economically sustainable, and suitable for each country-specific situation requires continuous effort.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2022.100479>.

## Funding

This study benefitted from a contract to IREC-UCLM from Ministry of Agriculture, Fisheries and Food, Madrid, Spain, and Research Grant PID2020-115046GB-I00 Ecología, salud pública y gestión del jabalí urbano, funded by the Spanish Ministerio de Ciencia e Innovación. P.P. received support from the MINECO-UCLM through an FPU grant (FPU16/00039) and from UNITO Dipartimento di Scienze Veterinarie (8B/2022/VET). B.C. is supported by the Fundação para a Ciência e Tecnologia, FCT (grant number 2020.04872.BD). M.E. received support from the MINECO through an FPU grant (FPU19/04651).

## CRediT authorship contribution statement

**P. Barroso:** Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **D. Relimpio:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **J.A. Zearra:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **J.J. Cerón:** Formal analysis, Investigation, Methodology, Resources, Validation, Writing – review & editing. **P. Palencia:** Conceptualization, Formal analysis, Methodology, Software, Visualization, Writing – review & editing. **B. Cardoso:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **E. Ferreras:** Investigation, Methodology, Visualization, Writing – review & editing. **M. Escobar:** Data curation, Investigation, Methodology, Visualization, Writing – review & editing. **G. Cáceres:** Conceptualization, Resources, Visualization, Writing – review & editing. **J.R. López-Olvera:** Conceptualization, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. **C. Gortázar:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors have no potential conflicts of interest to declare.

## Data availability

Data are available at the Zenodo repository ([doi:<https://doi.org/10.5281/zenodo.6855764>](https://doi.org/10.5281/zenodo.6855764)), including the metadata from camera trapping pictures (i.e. date, time and species), data on interspecific interactions and covariables to REM calculations in Excel files.

## Acknowledgement

We are grateful to the managers and technicians of the study sites, along with the hunters and vets who helped us access the hunted wild boar for sampling. The authors are grateful to Ajuntament de Barcelona, who supported field sampling through the contracts 13/051, 15/0174, 16/0243 and 16/0243-00-PR/01. We also thank for their collaboration: Y.F., A.G. and C.R. and JCCM staff in the province of Ciudad Real, J.A.M. in Doñana National Park, ARRECAL Huesca and M-C.A./D-F.L. in the province of Huesca, to M.M. in the area of Galicia, to F.G., J.M. and the park rangers of Redes Natural Park in Asturias, to B.C. in the area of Sierra de Gredos Regional Park, and finally to F.E. and C.M. in Murcia. We would also like to thank our colleagues from IREC for their help during fieldwork and data processing, as well as to all collaborating

National and Regional Veterinary Services and Wildlife Services.

## References

- [1] C. Gortázar, J.F. Ruiz-Fons, U. Höfle, Infections shared with wildlife: an updated perspective, *Eur. J. Wildl. Res.* 62 (2016) 511–525, <https://doi.org/10.1007/s10344-016-1033-x>.
- [2] C. Gortázar, P. Acevedo, F. Ruiz-Fons, J. Vicente, Disease risks and overabundance of game species, *Eur. J. Wildl. Res.* 52 (2006) 81–87, <https://doi.org/10.1007/s10344-005-0022-2>.
- [3] K.E. Jones, N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman, P. Daszak, Global trends in emerging infectious diseases, *Nature*. 451 (2008) 990–993, <https://doi.org/10.1038/nature06536>.
- [4] B. Cardoso, I. García-Bocanegra, P. Acevedo, G. Cáceres, P.C. Alves, C. Gortázar, Stepping up from wildlife disease surveillance to integrated wildlife monitoring in Europe, *Res. Vet. Sci.* 144 (2021) 149–156, <https://doi.org/10.1016/j.rvsc.2021.11.003>.
- [5] C. Machalaba, M. Uhart, M.-P. Ryser-Degiorgis, W.B. Karesh, Gaps in health security related to wildlife and environment affecting pandemic prevention and preparedness, 2007–2020, *Bull. World Health Organ.* 99 (2021) 342–350, <https://doi.org/10.2471/BLT.20.272690>.
- [6] B. Lawson, A. Neimanis, A. Lavazza, J.R. López-Olvera, P. Tavernier, C. Billinis, J. P. Duff, D.T. Mladenov, J.M. Rijks, S. Savić, G. Wibbelt, M.-P. Ryser-Degiorgis, T. Kuiken, How to start up a National Wildlife Health Surveillance Programme, *Animals*. 11 (2021) 2543, <https://doi.org/10.3390/ani11092543>.
- [7] P. Barroso, P. Acevedo, J. Vicente, The importance of long-term studies on wildlife diseases and their interfaces with humans and domestic animals: a review, *Transbound. Emerg. Dis.* 68 (2020) 1895–1909, <https://doi.org/10.1111/tbed.13916>.
- [8] J. Vicente, M. Apollonio, J.A. Blanco-Aguiar, T. Borowik, F. Brivio, J. Casae, S. Croft, G. Ericsson, E. Ferroglio, D. Gavier-Widen, C. Gortázar, P.A. Jansen, O. Keuling, R. Kowalczyk, K. Petrovic, R. Plhal, T. Podgórska, M. Sange, M. Scandura, K. Schmidt, G.C. Smith, R. Soriguer, H.-H. Thulke, S. Zanet, P. Acevedo, Science-based wildlife disease response, *Science*. 364 (2019) 943–944, <https://doi.org/10.1126/science.aax4310>.
- [9] C. Gortázar, P. Barroso, R. Nova, G. Cáceres, The role of wildlife in the epidemiology and control of foot-and-mouth-disease and similar transboundary (FAST) animal diseases: a review, *Transbound. Emerg. Dis.* 69 (2021) 2462–2473, <https://doi.org/10.1111/tbed.14235>.
- [10] F. Cecilianni, J.J. Ceron, P.D. Eckersall, H. Sauerwein, Acute phase proteins in ruminants, *J. Proteome* 75 (2012) 4207–4231, <https://doi.org/10.1016/j.jprot.2012.04.004>.
- [11] M.D. Contreras-Aguilar, A. Tvarijonaviciute, I. Monkoviciene, M. Martín-Cuervo, L. G. González-Arostegui, L. Franco-Martínez, J.J. Cerón, F. Tecles, D. Escribano, Characterization of total adenosine deaminase activity (ADA) and its isoenzymes in saliva and serum in health and inflammatory conditions in four different species: an analytical and clinical validation pilot study, *BMC Vet. Res.* 16 (2020) 384, <https://doi.org/10.1186/s12917-020-02574-2>.
- [12] J. Vicente, J. Martínez-Guijosa, A. Tvarijonaviciute, I.G. Fernandez-de Mera, C. Gortázar, J.J. Ceron, S. Martínez-Subiela, Serum haptoglobin response in red deer naturally infected with tuberculosis, *Comp. Immunol. Microbiol. Infect. Dis.* 64 (2019) 25–30, <https://doi.org/10.1016/j.cimid.2019.01.021>.
- [13] MAPAMA, Plan de Actuación sobre Tuberculosis en Especies Silvestres (PATUBES), Madrid, Spain. [https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/patubes2017\\_3\\_tcm30-378321.pdf](https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/patubes2017_3_tcm30-378321.pdf), 2017.
- [14] F. Ruiz-Fons, A review of the current status of relevant zoonotic pathogens in wild swine (*Sus scrofa*) populations: changes modulating the risk of transmission to humans, *Transbound. Emerg. Dis.* 64 (2017) 68–88, <https://doi.org/10.1111/tbed.12369>.
- [15] P. Palencia, J.M. Rowcliffe, J. Vicente, P. Acevedo, Assessing the camera trap methodologies used to estimate density of unmarked populations, *J. Appl. Ecol.* 58 (2021) 1583–1592, <https://doi.org/10.1111/1365-2664.13913>.
- [16] P. Palencia, P. Barroso, J. Vicente, T.R. Hofmeester, J. Ferreres, P. Acevedo, Random encounter model is a reliable method for estimating population density of multiple species using camera traps, *Remote Sens. Ecol. Conserv.* 8 (2022) 670–682, <https://doi.org/10.1002/rse2.269>.
- [17] J.M. Rowcliffe, J. Field, S.T. Turvey, C. Carbone, Estimating animal density using camera traps without the need for individual recognition, *J. Appl. Ecol.* 45 (2008) 1228–1236, <https://doi.org/10.1111/j.1365-2664.2008.01473.x>.
- [18] P. Palencia, J. Fernández-López, J. Vicente, P. Acevedo, Innovations in movement and behavioural ecology from camera traps: day range as model parameter, *Methods Ecol. Evol.* 12 (2021) 1201–1212, <https://doi.org/10.1111/2041-210X.13609>.
- [19] P. Palencia, Trappingmotion: Integrate camera-trapping in movement and behavioural studies, R package version 0.1.1 (2020). <https://github.com/PabloPalencia/trappingmotion> (accessed January 13, 2022).
- [20] J.M. Rowcliffe, Camtools. <https://github.com/MarcusRowcliffe/camtools>, 2022.
- [21] T.G. O'Brien, Abundance, density and relative abundance: A conceptual framework, in: *Camera Traps Anim. Ecol.*, Springer, Japan, Tokyo, 2011, pp. 71–96, [https://doi.org/10.1007/978-4-431-99495-4\\_6](https://doi.org/10.1007/978-4-431-99495-4_6).
- [22] E. Kukielka, J.A. Barasona, C.E. Cowie, J.A. Drewe, C. Gortazar, I. Cotarelo, J. Vicente, Spatial and temporal interactions between livestock and wildlife in south Central Spain assessed by camera traps, *Prev. Vet. Med.* 112 (2013) 213–221, <https://doi.org/10.1016/j.prevetmed.2013.08.008>.

- [23] J. Niedballa, A. Wilting, R. Sollmann, H. Hofer, A. Courtiol, Assessing analytical methods for detecting spatiotemporal interactions between species from camera trapping data, remote Sens. Ecol. Conserv. 5 (2019) 272–285, <https://doi.org/10.1002/rse2.107>.
- [24] G. Csardi, T. Nepusz, The igraph software package for complex network research, Inter. J. Compl. Syst. 1695 (2006) 1–9.
- [25] B. Martínez-López, A.M. Pérez, J.M. Sánchez-Vizcaíno, Social network analysis. Review of general concepts and use in preventive veterinary medicine, Transbound. Emerg. Dis. 56 (2009) 109–120, <https://doi.org/10.1111/j.1865-1682.2009.01073.x>.
- [26] A. Arenas-Montes, I. García-Bocanegra, J. Paniagua, J.J. Franco, F. Miró, M. Fernández-Morente, A. Carbonero, A. Arenas, Blood sampling by puncture in the cavernous sinus from hunted wild boar, Eur. J. Wildl. Res. 59 (2013) 299–303, <https://doi.org/10.1007/s10344-013-0701-3>.
- [27] P.C. Jocelyn, Spectrophotometric assay of thiols, Methods Enzymol. 143 (1987) 44–67, [https://doi.org/10.1016/0076-6879\(87\)43013-9](https://doi.org/10.1016/0076-6879(87)43013-9).
- [28] A. Tvarijonaviciute, F. Teclie, M. Caldin, S. Tasca, J. Cerón, Validation of spectrophotometric assays for serum paraoxonase type-1 measurement in dogs, Am. J. Vet. Res. 73 (2012) 34–41, <https://doi.org/10.2460/ajvr.73.1.34>.
- [29] CNIG, Corine Land Cover (España), <https://land.copernicus.eu/pan-european/corine-land-cover>, 2018 (accessed October 21, 2021).
- [30] QGIS Development Team, QGIS Geographic Information System. Open Source Geospatial Foundation Project. <https://qgis.org/es/site/>, 2019 (accessed October 1, 2019).
- [31] O.J. Dunn, Multiple comparisons using rank sums, Technometrics. 6 (1964) 241–252.
- [32] A.F. Zuur, E.N. Ieno, N.J. Walker, A.A. Saveliev, G.M. Smith, GLM and GAM for count data, in: Environ. Forum, 2009, pp. 209–243, [https://doi.org/10.1007/978-0-387-87458-6\\_9](https://doi.org/10.1007/978-0-387-87458-6_9).
- [33] K.P. Burnham, D.R. Anderson, Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, Springer, New York, 2002.
- [34] D. Bates, M. Maechler, B. Bolker, S. Walker, R.H.B. Chistensen, H. Singman, B. Dai, F. Sheipl, G. Grothendieck, O. Green, J. Fox, Linear Mixed-Effects Models Using “Eigen” and S4, 2019.
- [35] R Core Team, R: A Language and Environment for Statistical Computing. <https://www.r-project.org/>, 2019.
- [36] F. Keesing, R.S. Ostfeld, Dilution effects in disease ecology, Ecol. Lett. 24 (2021) 2490–2505, <https://doi.org/10.1111/ele.13875>.
- [37] J.A. Barasona, C. Gortázar, J. de la Fuente, J. Vicente, Host richness increases tuberculosis disease risk in game-managed areas, Microorganisms. 7 (2019) 182, <https://doi.org/10.3390/microorganisms7060182>.
- [38] D.J. Civitello, J. Cohen, H. Fatima, N.T. Halstead, L. Liriano, T.A. McMahon, C. N. Ortega, E.L. Sauer, T. Sehgal, S. Young, J.R. Rohr, Biodiversity inhibits parasites: broad evidence for the dilution effect, Proc. Natl. Acad. Sci. 112 (2015) 8667–8671, <https://doi.org/10.1073/pnas.1506279112>.
- [39] V. Naranjo, C. Gortázar, J. Vicente, J. de la Fuente, Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex, Vet. Microbiol. 127 (2008) 1–9, <https://doi.org/10.1016/j.vetmic.2007.10.002>.
- [40] N. Santos, V. Almeida, C. Gortázar, M. Correia-Neves, Patterns of *Mycobacterium tuberculosis*-complex excretion and characterization of super-shedders in naturally-infected wild boar and red deer, Vet. Res. 46 (2015) 129, <https://doi.org/10.1186/s13567-015-0270-4>.
- [41] P. Acevedo, J. Vicente, U. Höfle, J. Cassinello, F. Ruiz-Fons, C. Gortázar, Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment, Epidemiol. Infect. 135 (2007) 519–527, <https://doi.org/10.1017/S0950268806007059>.
- [42] M. Boadella, P. Acevedo, J. Vicente, G. Mentaberry, A. Balseiro, M.C. Arnal, D. Martínez, I. García-Bocanegra, C. Casal, J. Álvarez, Á. Oleaga, S. Lavín, M. Muñoz, J.L. Sáez-Llorente, J. de la Fuente, C. Gortázar, Spatio-temporal trends of Iberian wild boar contact with *Mycobacterium tuberculosis* complex detected by ELISA, Ecohealth. 8 (2011) 478–484, <https://doi.org/10.1007/s10393-011-0713-y>.
- [43] J. Vicente, F. Ruiz-Fons, D. Vidal, U. Höfle, P. Acevedo, D. Villanúa, I. G. Fernández-de-Mera, M.P. Martín, C. Gortázar, Serosurvey of Ajyeszky's disease virus infection in European wild boar in Spain, Vet. Rec. 156 (2005) 408–412, <https://doi.org/10.1136/vr.156.13.408>.
- [44] J. Vicente, J. Segalés, U. Höfle, M. Balasch, J. Plana-Durán, M. Domingo, C. Gortázar, Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (*Sus scrofa*), Vet. Res. 35 (2004) 243–253, <https://doi.org/10.1051/veteres:2004008>.
- [45] J. Martínez-Fernández, P. Ruiz-Benito, M.A. Zavala, Recent land cover changes in Spain across biogeographical regions and protection levels: implications for conservation policies, Land Use Policy 44 (2015) 62–75, <https://doi.org/10.1016/j.landusepol.2014.11.021>.
- [46] X. Fernández-Aguilar, M. Gottschalk, V. Aragon, J. Càmara, C. Ardanuy, R. Velarde, N. Galofré-Milà, R. Castillo-Contreras, J.R. López-Olvera, G. Mentaberry, A. Colom-Cadena, S. Lavin, O. Cabezón, Urban wild boars and risk for zoonotic *Streptococcus suis*, Spain, Emerg. Infect. Dis. 24 (2018) 1083–1086, <https://doi.org/10.3201/eid2406.171271>.
- [47] H. Wang, R. Castillo-Contreras, F. Sagutti, J.R. López-Olvera, M. Karlsson, G. Mentaberry, M. Lindh, J. Serra-Cobo, H. Norder, Genetically similar hepatitis E virus strains infect both humans and wild boars in the Barcelona area, Spain, and Sweden, Transbound. Emerg. Dis. 66 (2019) 978–985, <https://doi.org/10.1111/tbed.13115>.
- [48] V. Lizana, C. Gortázar, A. Muniesa, Ó. Cabezón, A. Martí-Marco, J. López-Ramon, J. Cardells, Human and environmental factors driving *Toxoplasma gondii* prevalence in wild boar (*Sus scrofa*), Res. Vet. Sci. 141 (2021) 56–62, <https://doi.org/10.1016/j.rvsc.2021.10.007>.
- [49] R. Triguero-Ocaña, B. Martínez-López, J. Vicente, J.A. Barasona, J. Martínez-Guijosa, P. Acevedo, Dynamic network of interactions in the wildlife-livestock interface in mediterranean Spain: an epidemiological point of view, Pathogens. 9 (2020) 1–16, <https://doi.org/10.3390/pathogens9020120>.
- [50] C.F. Pierce, V.R. Brown, S.C. Olsen, P. Boggiatto, K. Pedersen, R.S. Miller, S. E. Speidel, T.J. Smyser, Loci associated with antibody response in feral swine (*Sus scrofa*) infected with *Brucella suis*, Front. Vet. Sci. 7 (2020), 554674, <https://doi.org/10.3389/fvets.2020.554674>.
- [51] A. Ráez-Bravo, J.E. Granados, J.J. Cerón, F.J. Cano-Manuel, P. Fandos, J.M. Pérez, J. Espinosa, R.C. Sorribes, J.R. López-Olvera, Acute phase proteins increase with sarcoptic mange status and severity in Iberian ibex (*Capra pyrenaica*, Schinz 1838), Parasitol. Res. 114 (2015) 4005–4010, <https://doi.org/10.1007/s00436-015-4628-3>.
- [52] P.L. McCarthy, A.L. Frank, R.C. Ablow, S.J. Masters, T.F. Dolan, Value of the C-reactive protein test in the differentiation of bacterial and viral pneumonia, J. Pediatr. 92 (1978) 454–456, [https://doi.org/10.1016/S0022-3476\(78\)80448-X](https://doi.org/10.1016/S0022-3476(78)80448-X).
- [53] V. Chandra, A. Kar-Roy, S. Kumari, S. Mayor, S. Jameel, The hepatitis E virus ORF3 protein modulates epidermal growth factor receptor trafficking, STAT3 translocation, and the acute-phase response, J. Virol. 82 (2008) 7100–7110, <https://doi.org/10.1128/JVI.00403-08>.
- [54] A. Parra, A. García, N.F. Inglis, A. Tato, J.M. Alonso, M. Hermoso de Mendoza, J. Hermoso de Mendoza, J. Larrasa, An epidemiological evaluation of *Mycobacterium bovis* infections in wild game animals of the Spanish Mediterranean ecosystem, Res. Vet. Sci. 80 (2006) 140–146, <https://doi.org/10.1016/j.rvsc.2005.05.010>.
- [55] B. Wells, G.T. Innocent, P.D. Eckersall, E. McCulloch, A.J. Nisbet, S.T. Burgess, Two major ruminant acute phase proteins, haptoglobin and serum amyloid a, as serum biomarkers during active sheep scab infestation, Vet. Res. 44 (2013) 103, <https://doi.org/10.1186/1297-9716-44-103>.
- [56] J.A. Barasona, J. Vicente, I. Díez-Delgado, J. Aznar, C. Gortázar, M.J. Torres, Environmental presence of *Mycobacterium tuberculosis* complex in aggregation points at the wildlife/livestock Interface, Transbound. Emerg. Dis. 64 (2017) 1148–1158, <https://doi.org/10.1111/tbed.12480>.
- [57] B. Martínez-López, A.M. Pérez, J.M. Sánchez-Vizcaíno, Social network analysis. Review of general concepts and use in preventive veterinary medicine, Transbound. Emerg. Dis. 56 (2009) 109–120, <https://doi.org/10.1111/j.1865-1682.2009.01073.x>.