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Seronegativity of Iberian ibex (*Capra pyrenaica*) against *Toxoplasma gondii* and *Neospora caninum* is consistent with eco-epidemiological and environmental features in Mediterranean mountainous areas

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ABSTRACT

Knowledge of pathogen epidemiological dynamics and habitat ecological features is essential for wildlife population and health monitoring and management. *Toxoplasma gondii* and *Neospora caninum* are two broadly distributed multi-host parasites that affect both wild and domestic animals and, in the case of *T. gondii*, cause zoonosis. This study reports the seroprevalence of both parasites in Iberian ibex (*Capra pyrenaica*), a mountain wild ruminant native to the Iberian Peninsula, from the Natural Space of Sierra Nevada (NSSN) in southeastern Spain. Serum from 146 Iberian ibexes were analysed using two in-house ELISA techniques. The positive and doubtful sera were further checked by Western Blot (WB). Seventeen ibexes (11.6 %; 95 % confidence interval 6.4–16.7) were positive for *T. gondii* and seven (4.8 %; 95 % confidence interval 1.3–8.2) for *N. caninum*. However, no sera were positive to *T. gondii* nor to *N. caninum* by WB. Using at least two different serological techniques is recommended when they are not validated for the target host species. The NSSN is a hypoendemic area for *T. gondii* and *N. caninum*, probably determined by the reduced abundance and restricted distribution of their definitive hosts. This would explain the hypoendemic situation in the NSSN and the lack of specific antibodies against these two parasites in the Iberian ibex population. This eco-epidemiological scenario can be challenged by climate and anthropogenic changes, recommending long-term monitoring Iberian ibex population and health, both as a conservation measure for the species and as an indicator of the potential impact of global change on high mountain ecosystems.

1. Introduction

Wildlife shares pathogens with humans and domestic animals, which is relevant to public health and animal health as well as to livestock production (Daszak et al., 2000; Gortázar et al., 2016; Jones et al., 2013). Moreover, the spread of such multi-host pathogens can have negative effects for the conservation of wildlife populations and, consequently, should be a key issue in the management of wild species (Caron et al., 2015; Fagre et al., 2022). Integrating wildlife health monitoring and management within the One Health approach implies facing a complex and multifactorial issue that requires as accurate knowledge as possible about animal species under study, including the demographic and ecological features of the population, as well as the eco-epidemiological dynamics of pathogens (Barroso et al., 2022; Cable

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et al., 2017; Wilson et al., 2024).

Iberian ibex (*Capra pyrenaica*) is a medium-sized mountain ungulate endemic to the Iberian Peninsula and the northern Pyrenees in France (Pérez et al., 2024). While overall population and geographic distribution range are increasing, leading to coalescence of formerly unconnected population nuclei (Pérez et al., 2024), the populations of Maestrazgo in northeastern Spain and Sierra Nevada in southeastern Spain remain as the more abundant (Granados et al., 2020). The Natural Space of Sierra Nevada (NSSN; Granados et al., 2020), a protected isolated mountainous massif in southeastern Spain and one of the most important European hotspots for biodiversity, hosts the Iberian ibex population of Sierra Nevada, which spreads outside the limits of the protected area (Granados et al., 2020). High mountain ecosystems are among those suffering the greatest impact from human-driven global change (Vitousek, 1994), whose effects are being monitored in the NSSN since 2007 (Zamora et al., 2016). Global change (both climatic and anthropic) is affecting both disease epidemiology and wild ungulate population demography (Altizer et al., 2013; Brooks and Hoberg, 2007; Cable et al., 2017; White et al., 2018). Thus, assessing and monitoring the dynamics of pathogens within the Sierra Nevada Iberian ibex population is not only relevant for its conservation and potential involvement in the maintenance and spread of multi-host pathogens, but also as an indicator of global change in the southernmost European mountain range (López-Olvera et al., 2024; Valldeperes et al., 2023).

Toxoplasmosis and neosporosis, caused respectively by the Apicomplexa parasites *Toxoplasma gondii* and *Neospora caninum*, are two widely distributed diseases. Both have similar life cycles, with warm-blooded vertebrates as intermediate hosts, and with felids as the definitive hosts for *T. gondii* (Dubey, 2010) and canids for *N. caninum* (Bandelj et al., 2023). While both parasites have significant impact on livestock, particularly in ruminants, only *T. gondii* is zoonotic (Dubey, 2010; Dubey and Schares, 2011; Dubey et al., 2007). A wide range of intermediate hosts has been described for *T. gondii*, from bird to mammal species including humans (Dubey, 2010), whereas the number of known intermediate hosts for *N. caninum* is more restricted, including bovids, sheep, goats, horses, cats and other domestic and wildlife species (Almería, 2013; Donahoe et al., 2015; Dubey and Schares, 2011; Dubey et al., 1999; Lindsay and Dubey, 2020). Ruminants become infected mainly through the ingestion of *T. gondii* and *N. caninum* sporulated oocysts from the environment, after being excreted with the faeces of felids and canids, their respective definitive hosts (Dubey et al., 2007; Zhu et al., 2022).

The monitoring and potential further management of wild populations implies the reliable detection of individuals affected by and/or that have been in contact with pathogens (Thrusfield et al., 2018). Serological tests are the most used, since they allow widespread analysis, they are cost-effective and easy to use for large sample sizes (Liu et al., 2015; Wyrosdick and Schaefer, 2015). However, there are few validated techniques for wildlife species, which makes difficult to establish a cut-off threshold to discriminate positive and negative reactions (Gondim et al., 2017; Huertas-López et al., 2023, 2024). On the other hand, environmental and ecological factors are key for pathogen-host interaction, conditioning the presence or absence of pathogens (Gilbert et al., 2013). Therefore, the conclusions drawn from pathogen diagnosis in wild species should also be based on the ecological and epidemiological features of the study area (Cable et al., 2017; Giraudoux et al., 2008), avoiding inaccurate understanding of pathogen transmission, persistence and spread and, consequently, obtaining a more comprehensive overview of the eco-epidemiological landscape (Suh et al., 2024).

In wild and domestic species, the diagnosis of *T. gondii* and *N. caninum* infection is usually based on serological techniques, mainly indirect fluorescent antibody tests (IFAT), enzyme-linked immunoassays (ELISA) and agglutination techniques (Huertas-López et al., 2024; Liu et al., 2015; Sinnott et al., 2017; Wyrosdick and Schaefer, 2015). However, these techniques have limitations particularly relevant in wildlife, such as the lack of species-specific conjugates (in ELISA and IFAT techniques); the subjective interpretation of the results (in IFAT and agglutination techniques); the labour-intensive nature and the time required to perform the technique (e.g. Western Blot -WB-); the cross-reactivity with other Apicomplexa parasites; and the variable sensitivity and specificity depending on the antigen used (Anderson et al., 2007; Dubey et al., 2009; Gondim et al., 2017; Huertas-López et al., 2023, 2024; Liu et al., 2015; López Ureña et al., 2023; Sinnott et al., 2017; Wyrosdick and Schaefer, 2015).

The seroprevalence of *T. gondii* and *N. caninum* has been studied in different Iberian ibex populations from Spain, but not in the metapopulation of the Sierra Nevada massif. Specifically, the seroprevalences against *T. gondii* reported in this wild ruminant using modified agglutination tests (MAT) and/or ELISA range from 2.9 % to 27.5 % (Almería et al., 2018, 2021; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012; Gauss et al., 2006). The seroprevalences of *N. caninum* reported in Iberian ibex by IFAT and ELISA range from 0.0 % to 13 % (Almería et al., 2007; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012). However, none of these techniques has been validated for Iberian ibex. Moreover, they have generally been used as a single technique in the serological studies carried out to date, without contrasting the results with other techniques.

This study has consequently two objectives: first, assessing the presence of antibodies against *T. gondii* and *N. caninum* in the Iberian ibex population of the Sierra Nevada massif using two serological techniques, namely ELISA and WB; and second, taking advantage of the use of both serological tests on the same sample set, discuss whether the eco-epidemiological interpretation of the serological results obtained with ELISA techniques would have been different if these results had not been compared with those found by the WB technique.

2. Material and methods

2.1. Ethics statement

This study complied with all Andalusian, Spanish and European legal requirements and guidelines regarding experimentation and animal welfare. Handling procedures and sampling were designed to reduce stress and minimize the impact on the health of the animals. The sampling of the Iberian ibexes formed part of the regular population monitoring of the species in Sierra Nevada (Granados et al., 2020), and therefore the Animal Experimentation Ethics Committee (CEEA) of the University of Murcia concluded that approval was not required, since no ibex was captured, handled nor sampled specifically for the purpose of this study.

2.2. Study area

Located in the centre of the Baetic mountain range in southeastern Spain (37° 12′ 40” N, 3° 32′ 51” O to 36° 56′ 26” N, 2° 39′ 50” O), the NSSN spreads over 172,318 ha, altitude ranging from 300 to 3482 m above sea level. The NSSN is formed by the core National Park (85,883 ha) and the surrounding Natural Park (86,355 ha). The climate is generally Mediterranean and cold continental in the highest areas due to the altitude, with seasonal climatic variations (Junta de Andalucía, 2020). Iberian ibex is the most abundant ungulate species in the NSSN (Granados et al., 2020), although wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) also exist in lower densities (Granados and Cano-Manuel, 2016; Granados et al., 2021).

2.3. Sample collection

Blood samples without anticoagulant were collected from the jugular vein of 146 free-ranging Iberian ibexes, captured by teleanaesthesia with xylazine and ketamine (Casas-Díaz et al., 2011) or drive nets (López-Olvera et al., 2009) in the NSSN from 2010 to 2015 (Table 1).

Table 1
Sex and age class of the Iberian ibexes sampled from the metapopulation of the NSSN and surrounding areas (southeastern Spain).

	Age class	Male	Female	Total
Age	Kid (<1 year)	3	2	5 (3.4 %)
	Young (1–2 years)	11	7	18 (12.3 %)
	Subadult (3–4 years)	16	4	20 (13.7 %)
	Adult (5–8 years)	67	20	87 (59.6 %)
	Old (> 8 years)	14	2	16 (11.0 %)
Total		111 (76.0 %)	35 (24.0 %)	146 (100.0 %)

Taking into account the maximum seroprevalence ever reported for *T. gondii* and *N. caninum* in Iberian ibex (27.5 % for *T. gondii*, García-Bocanegra et al., 2012; Valldeperes et al., 2023), the sample size of the study allowed to estimate the prevalence with a 95 % confidence level and a 3.5 % precision (Pourhoseingholi et al., 2013). Most of the ibexes were captured within the NSSN boundaries, except for some ibexes that came from neighbouring mountainous areas and are, therefore, part of the same Iberian ibex metapopulation (Pérez et al., 2024; Fig. 1). Age class and sex of the individuals captured was assessed following previously reported criteria (Fandos, 1991).

2.4. Laboratory analyses

The blood samples were allowed to clot at room temperature. The sera were obtained by centrifugation at 1200*g* for 15 min and stored at −20 °C until analysis. Two in-house ELISA techniques were first used for screening of antibodies against *T. gondii* and *N. caninum*, respectively, according to previously described protocols (Gutiérrez-Expósito et al., 2013) with specific modifications. The plates were coated using soluble extract of sonicated tachyzoites of *T. gondii* strain *Tg*-ME49 (Chávez-Velásquez et al., 2005) and *N. caninum* strain *Nc*-1 (Álvarez-García et al., 2002). Sera from domestic goats and sheep where the absence or presence of the respective parasite had been diagnosed were used as negative and positive controls, respectively. After blocking the plates with 3 % bovine serum albumin (Fraction V, Roche®), for each parasite two 1:100 dilution aliquots of all the sera were incubated at 37 °C for one hour. Then, recombinant peroxidase-labelled protein G (Sigma®) was used as conjugate at 1:2000 dilution. After adding ABTS® substrate (Roche Diagnostics), the optical density (OD) of the colorimetric reaction was measured at a wavelength of 405 nm. The OD was transformed into a relative index percent (RIPC) by the following formula: RIPC = (OD_{405} sample – OD_{405} negative control) / (OD_{405} positive control – OD_{405} negative control) x 100. The cut-off was established as mean RIPC value of the negative controls plus three-fold the standard deviation of this value.

Since the in-house ELISA techniques used have not been validated for Iberian ibex, the sera considered as positive and/or doubtful by the ELISA were subsequently analysed by WB. Membranes and immunotransference were performed in 15 % polyacrylamide gel under reducing conditions following previously reported methodology (Fernández-García et al., 2009). The same strains and sera used in the in-house ELISAs were employed as antigens and controls, respectively, and a previously reported protocol was followed (Gutiérrez-Expósito et al., 2013). After blockade with tris-buffered saline with 0.05 % Tween® 20 detergent (TBST, Sigma-Aldrich®) plus 5 % skimmed milk, the membranes were incubated for one hour with a 1:20 dilution of the sera. Then, after three washes with TBST, recombinant peroxidase-labelled protein G (Sigma®) at 1:150 dilution was added. After a new one hour-incubation, three washes with TBST and two with TBS (without Tween® 20 detergent) were performed, and a solution based on 4-chloro-1-naphthol (Thermo Scientific) was used to reveal the strips. The reaction was stopped with ultra-pure water based on the reaction that occurs in the positive controls. Finally, the strips were scanned on the GS-800 Calibrated Densitometer scanner (Bio-Rad). For *T. gondii* WB, positivity criteria were based on the detection of reaction against 22–24, 30, and 38–40 kDa tachyzoite immunodominant antigens (IDAs; Chávez-Velásquez et al., 2005). For *N. caninum* WB, positivity criteria were based in the detection of reaction against 17–18, 34–35, 37, and 60–62 kDa tachyzoite IDAs. A serum was considered positive when at least the 17–18 and/or the 37 kDa IDAs were detected (Álvarez-García et al., 2002).

3. Results

Seventeen out of the 146 sera (11.6 %, central confidence interval 95 % 6.4–16.8) were positive according to the *T. gondii* ELISA, while seven of the 146 sera (4.8 %, central confidence interval 95 % 1.3–8.3) yielded positive results in the *N. caninum* ELISA. However, no positive sera were detected by WB for neither *T. gondii* nor *N. caninum* (Supplementary data).

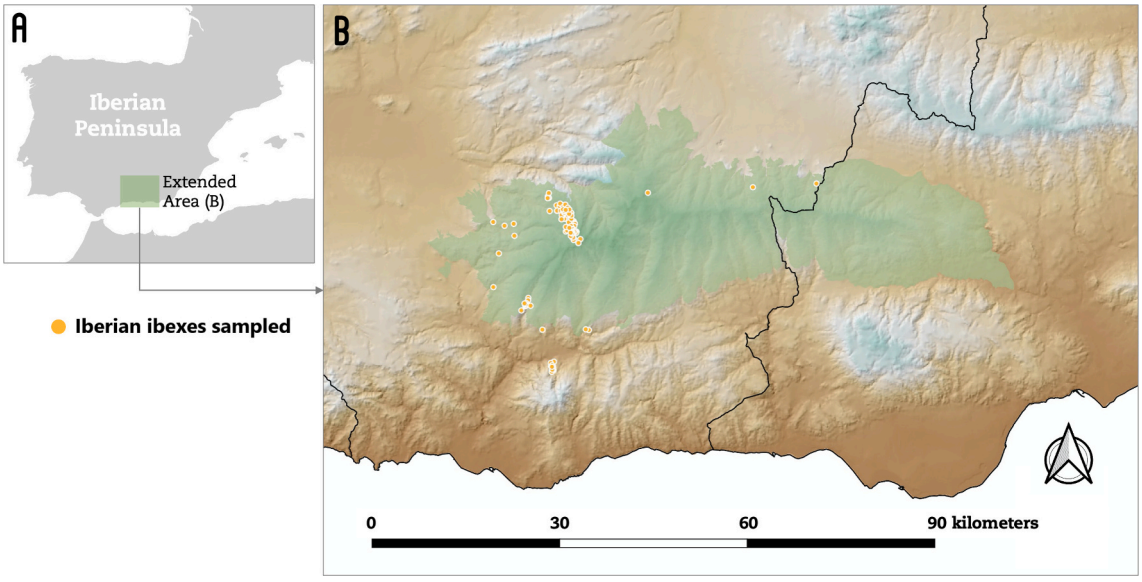

Fig. 1. Location of the captures of the 146 Iberian ibexes sampled (yellow spots on the map). The green area is the Natural Space of Sierra Nevada (NSSN, southeastern Spain). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This study reports for the first time the prevalence of antibodies against *T. gondii* and *N. caninum* in the Iberian ibex population of the NSSN. To the authors' knowledge, this is also the first study in this species using WB to check ELISA positive results against these two Apicomplexa parasites. The *T. gondii* seroprevalences previously described for free-ranging wild ruminants from the Iberian Peninsula, including Iberian ibex, red deer, fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), mouflon (*Ovis gmelini*), southern chamois (*Rupicapra pyrenaica*) and aoudad (*Ammotragus lervia*) range from 1.5 % to 39.6 % (Almería et al., 2018, 2021; Calero-Bernal et al., 2020; Candela et al., 2009; Castro-Scholten et al., 2021; Gamarra et al., 2008; García-Bocanegra et al., 2012; Gauss et al., 2006; Panadero et al., 2010; San Miguel et al., 2016), with overall lower values ranging from 0.0 % to 12.9 % for *N. caninum* (Almería et al., 2007; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012; Panadero et al., 2010; San Miguel et al., 2016). Specifically, the serological prevalences of *T. gondii* previously reported in Iberian ibex using either MAT, IFAT and/or ELISA (2.9 % to 27.5 %; Almería et al., 2007, 2018, 2021; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012; González-Barrio et al., 2024) fall within the lower part of the overall range described for all the ruminant species in the Iberian Peninsula, whereas the seroprevalences for *N. caninum* previously reported in Iberian ibex (0.0 % to 12.9 %; Almería et al., 2007; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012) cover the whole range previously reported for all the wild ruminant species from the Iberian Peninsula.

The seroprevalences of both parasites in the Iberian ibex population of the NSSN detected by ELISA are, therefore, within the values previously described for this wild ruminant in Spain. However, the seropositive Iberian ibexes for *T. gondii* and *N. caninum* by ELISA were WB seronegative. Both techniques have been recently used for the detection of anti-*T. gondii* and anti-*N. caninum* antibodies in domestic goat, using the same native antigens and a monoclonal anti-goat/sheep immunoglobulin G (IgG) secondary antibody (Huertas-López et al., 2021; Sánchez-Sánchez et al., 2021), but despite the phylogenetic proximity between both species, ELISA and WB are not currently validated for Iberian ibex. Protein G, as used in this study, has shown high sensitivity and specificity for the detection of Apicomplexa parasite infections in sheep (Sánchez-Sánchez et al., 2018), similar to anti-sheep IgG secondary antibody (Sánchez-Sánchez et al., 2021), and has also been used for the diagnosis of Apicomplexa parasite infections in other wild ruminants, such as Nubian ibex (*Capra nubiana*) and mouflon (*Ovis orientalis*, Mazuz et al., 2018). It is advisable to use a strict positivity criterion, considering as positive only those samples that were positive by at least two techniques (Donahoe et al., 2015). Therefore, none of the sampled ibexes should be considered seropositive to none of the two parasites investigated. While WB is considered a more reliable technique to detect specific antibodies against both parasites (López Ureña et al., 2023; Mazuz et al., 2018), it is a laborious technique not routinely used in the diagnosis of *T. gondii* and *N. caninum* in wild ungulates (Anderson et al., 2007; Chávez-Velásquez et al., 2005; Dubey et al., 2009;). ELISA false positives can occur because of cross reaction with other Apicomplexa species (Huertas-López et al., 2021; Gondim et al., 2017) or because interferences due to serum quality (Gutiérrez-Expósito et al., 2013). These results reinforce the need to confirm the results obtained by MAT, IFAT and ELISA with other techniques with higher specificity, such as WB, when analysing samples from wild host species where the techniques have not been validated, as the Iberian ibex. The lack of confirmation through WB of the sera detected as positive by the ELISA raises concern about the meaning and interpretation of the seroprevalences of *T. gondii* and *N. caninum* described in previous studies in Iberian ibexes from other mountainous areas using only one laboratory diagnostic technique (Almería et al., 2007, 2018, 2021; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012).

According to the more reliable WB results, the NSSN is a hypoendemic area for *T. gondii* and *N. caninum*, which is consistent with the environmental, ecological and epidemiological characteristics of mountain ecosystem. Environmentally, the prolonged periods of low temperatures in the snowy period, the presence of sparse vegetation cover that favours solar action, and the dryness and insolation of rocky areas may reduce the survival of *T. gondii* and *N. caninum* oocysts (Dubey et al., 2007; Hershey and McGregor, 1987; Shapiro et al., 2019). Eco-epidemiologically, the presence of definitive hosts for *T. gondii* and *N. caninum* in mountain ecosystems determines the maintenance of the sylvatic cycle of these parasites. In the NSSN, the only definitive host species present are European wildcat (*Felis silvestris*) and domestic cat (*Felis catus*) for *T. gondii* (Dubey, 2010) and domestic dog for *N. caninum* (Dubey et al., 2007). However, the NSSN has one of the lowest wildcat population densities in southern Spain (Gil-Sánchez et al., 2015, 2020). In addition, this felid mostly occupies lower altitudes than the Iberian ibex (Gil-Sánchez et al., 2015; Moleón and Gil-Sánchez, 2003), thereby reducing the likelihood of Iberian ibex exposure to infective *T. gondii* oocysts shed by European wildcats. This pattern of altitudinal gradient has been observed in other mountainous European areas with low *T. gondii* seroprevalence in wild ruminants (Ferroglio et al., 2014). Conversely, domestic cat contributes most to the dispersal of *T. gondii* oocysts due to its high abundance and close association with humanized areas (VanWormer et al., 2013; Zhu et al., 2022). Nevertheless, while within the NSSN domestic cats are abundant, their presence is limited to human settlements, and they are rarely found far from these areas. Similarly, the presence of domestic dogs is mostly restricted to inhabited areas. Consequently, both domestic species are predominantly linked to humanized areas and not widely distributed throughout the NSSN. Altogether, the environmental conditions for oocyst survival at high altitudes and the reduced abundance and restricted distribution of the oocyst-shedding definitive hosts for *T. gondii* and *N. caninum* probably explain the hypoendemic status and, consequently, the lack of detection of specific antibodies against these two parasites in the NSSN Iberian ibex population.

Global change may shift such hypoendemic condition for *T. gondii* and *N. caninum* in the NSSN and Mediterranean mountain areas in general (Brooks and Hoberg, 2007; Gsell et al., 2023) through (1) anthropic-driven increase in the presence of the domestic definitive hosts (dogs and cats) associated with human presence (either as visitors or settlers creating new human population nuclei), favouring the environmental propagation of oocysts (Ballash et al., 2019; Barros et al., 2018; Barroso et al., 2020; Costanzi et al., 2021; VanWormer et al., 2013; Wilson et al., 2024); and (2) climate warning increasing temperature and decreasing snow cover (Jiménez et al., 2019), allowing the survival of infective stages in the environment (Suh et al., 2024). The entry of these parasites in the NSSN could demographically impact the Iberian ibex population, since the lack of previous contact with infective Apicomplexa oocysts could lead to high disease rates and reproductive failure, as reported in other wild ruminant species (Donahoe et al., 2015; Dubey et al., 2007, 2010; Patel et al., 2019; Soler et al., 2022). Moreover, such entry would build upon the current endemic presence of different pathogens, such as *Sarcoptes scabiei* and *Mycoplasma conjunctivae*, which already condition the ecology, epidemiology, and demography of the Iberian ibex population in the NSSN (López-Olvera et al., 2024; Pérez et al., 1997). Coinfection with different parasites could synergically worsen the health and demography of the host population, increasing its probability of extinction (Garrido-Amaro et al., 2023; Hananeh et al., 2022; Vaumourin et al., 2015). Therefore, monitoring *T. gondii* and *N. caninum* in the NSSN would serve to a double purpose: on the one hand, it would allow to detect potential ecosystem alterations induced by changes in human presence and activity within the global change monitoring at the NSSN (Granados and Cano-Manuel, 2016; Zamora et al., 2016); on the other hand, as a part of integrated wildlife monitoring (Barroso et al., 2023; McMahon et al., 2018), it would help to the early-detection and establishment of fast-response correction and management measures to protect the Iberian ibex population of the

NSSN in case of introduction of these two parasites.

5. Conclusions

This study provided the first reliable results for serological prevalence of *T. gondii* and *N. caninum* in the Iberian ibex population of the NSSN. While the parasites seem to be absent, the results previously published for other Iberian ibex populations should be geographically expanded and verified with more sensitive and specific methodologies (such as WB), to ascertain and more accurately know the eco-epidemiological status of *T. gondii* and *N. caninum* in the Iberian ibex metapopulation of the Iberian Peninsula. The absence of *T. gondii* and *N. caninum* suggests that including the surveillance of these parasites in the current global change monitoring of the NSSN would help to detect both ecosystem alterations and health risks for the Iberian ibex population.

CRediT authorship contribution statement

Alejandro Cano-Manuel: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **José Enrique Granados:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Gema Álvarez-García:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Ana Huertas-López:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Carlos Diezma-Díaz:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Francisco Javier Cano-Manuel:** Writing – review & editing. **Luis Miguel Ortega-Mora:** Writing – review & editing, Resources, Funding acquisition. **Paulino Fandos:** Writing – review & editing. **Gregorio Mentaberre:** Writing – review & editing, Investigation. **Jorge Ramón López-Olvera:** Writing – review & editing, Writing – original draft, Investigation. **Carlos Martínez-Carrasco:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2025.105530.

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