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

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ABSTRACT

Monitoring and management of wildlife health is a complex multifactorial issue that must be based on the most complete knowledge possible of the target animal species, including the epidemiological dynamics of the pathogens and the ecological characteristics of the habitat where the host population resides. *Toxoplasma gondii* and *Neospora caninum* are two multi-host parasites broadly distributed. They 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 wild ruminant native to the Iberian Peninsula that occupies mountain habitats. Specifically, 146 Iberian ibexes from the metapopulation distributed in the Natural Space of Sierra Nevada (NSSN), a protected area in southeastern Spain, were analysed using two in-house ELISA techniques based on soluble extract of sonicated tachyzoites of *T. gondii* (strain Tg-ME49) and *N. caninum* (strain Nc-1), respectively. Seventeen animals (11.6%; 95% confidence interval: 6.4-16.7) were positive for *T. gondii* and seven for *N. caninum* (4.8%; 1.3-8.2). Since in-house ELISA techniques are not validated for Iberian ibex, positive and doubtful sera were also checked by Western Blot (WB) to minimize the risk of false positives due to cross-reactivity between these and other Apicomplexa parasites. No positive sera were detected by WB to *T. gondii* nor to *N. caninum*. These results indicate the convenience of using at least two different serological techniques when serodiagnosis is based on non-validated techniques for the target host species, as Iberian ibex. Moreover, they show that NSSN is a hypoendemic area for *T. gondii* and *N. caninum*, which is consistent with the environmental, ecological and epidemiological conditions of the mountain habitat occupied by Iberian ibex. The presence in mountain ecosystems of the definitive hosts of these parasites is determinant for the maintenance of their sylvatic cycles. The reduced abundance of European wildcat (*Felis silvestris*) and the restricted distribution of domestic dogs and cats probably explain this hypoendemic situation in the NSSN and, consequently, the lack of specific antibodies against these two parasites in the Iberian ibex population. This eco-epidemiological scenario is susceptible to be modified by climate change or

anthropogenic causes, which suggests the convenience of monitoring the Iberian ibex population in the long-term, as a conservation measure for the species and as a potential indicator of the impact that global change may have on high mountain ecosystems.

Keywords: Eco-epidemiology; Iberian ibex; *Neospora caninum*; *Toxoplasma gondii*; Western blot

1. INTRODUCTION

Wildlife shares numerous 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; Karmacharya et al., 2024). 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 et al., 2017; Hudson et al., 2002; Karesh et al., 2012; McEachran et al., 2024; 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 (Acevedo and Cassinello, 2009; Granados et al., 2001; Manceau et al., 1999; Pérez et al., 2024). While overall population and geographic distribution range are increasing, leading to coalescence of formerly unconnected population nuclei (Pérez et al., 2002, 2024), the populations of Maestrazgo in northeastern Spain and Sierra Nevada in southeastern Spain remain as the more abundant (Angelone et al., 2018; Granados et

al., 2020; Manceau et al., 1999). 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 climate change, so the effects of global change on the NSSN are being monitored since 2007 (García-Del-Amo et al., 2023; Zamora et al., 2016). Such environmental changes, largely driven by human activity (Vitousek, 1994), can affect wild species of mountainous areas, including their demographic features, their reproduction and health, as has been observed in alpine ungulates, including the Iberian Ibex (Granados et al., 2020; White et al., 2018). Moreover, the climatic change also impacts on distribution of animal species (Abrahms et al., 2023; Pigot et al., 2023), community and food web interactions (Van Moorsel et al., 2023), and pathogen-host ecology, leading to new eco-epidemiological scenarios (Altizer et al., 2013; Bezerra-Santo et al., 2023; Brooks and Hoberg, 2007; Cable et al., 2017; Karmacharya et al, 2024; Rupasinghe et al., 2022; Van de Vuurst et al., 2023). Thus, assessing and monitoring the dynamics of pathogens within the Sierra Nevada Iberian ibex population is not only relevant for the conservation of this wild ruminant species and its potential involvement in the maintenance and spread of multi-host pathogens that also affect domestic animals and humans, but also as an indicator of global (both climatic and anthropic) 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), including the dog (*Canis lupus familiaris*), coyote (*Canis latrans*), grey wolves (*Canis lupus*), dingo (*Canis lupus dingo*) and golden jackal (*Canis aureus*). While both parasites have significant impact on livestock,

100 particularly in ruminants, only *T. gondii* is zoonotic (Benavides et al., 2022; Dubey, 2010; Dubey
101 and Schares, 2011; Dubey et al., 2007; McAllister et al., 1998; Tenter et al., 2000). A wide range
102 of intermediate hosts has been described for *T. gondii*, from bird to mammal species including
103 humans (Dubey, 2010), whereas the number of known intermediate hosts for *N. caninum* is more
104 restricted, including bovids, sheep, goats, horses, cats and other domestic and wildlife species
105 (Almería 2013; Donahoe et al., 2015; Dubey and Schares, 2011; Dubey et al., 1999; Lindsay and
106 Dubey, 2020). Ruminants become infected mainly through the ingestion of *T. gondii* and *N.*
107 *caninum* sporulated oocysts from the environment, after being excreted with the faeces of felids
108 and canids, their respective definitive hosts (Dubey et al., 2007; Zhu et al., 2022).

109 The monitoring and potential further management of wild populations implies the reliable
110 detection of individuals affected by and/or that have been in contact with pathogens (Thrusfield
111 et al., 2018). Serological tests are the most used, since they allow widespread analysis, they are
112 cost-effective and easy to use for large sample sizes (Liu et al., 2015; Wyrosdick and Schaefer,
113 2015). However, the interpretation of serological results requires prudence when inferring
114 conclusions about disease ecology, since cross-reactivity is often involved, there are few validated
115 techniques for wildlife species, and standardized protocols for sample collection and analysis are
116 scarce, which altogether makes difficult to establish a cut-off threshold to discriminate positive
117 and negative reactions (Gondim et al., 2017; González-Barrio et al., 2024; Huertas-López et al.,
118 2023, 2024; Jia et al., 2020;). On the other hand, environmental and ecological factors are key for
119 pathogen-host interaction, conditioning environmental gradients that may favour or hinder this
120 interaction and, consequently, the presence or absence of a given pathogen in a particular area
121 (Gilbert et al., 2013). Therefore, beyond the aforementioned methodological and analytical
122 restrictions, the conclusions drawn from pathogen diagnosis in wild species should also be based
123 on the ecological and epidemiological features of the study area (Cable et al., 2017; Giraudoux et
124 al., 2008), avoiding an inaccurate understanding of the patterns of pathogen transmission,

125 persistence and spread and, consequently, obtaining a more accurate overview of the eco-
126 epidemiological landscape (Suh et al., 2024).

127 In wild and domestic species, the diagnosis of *T. gondii* and *N. caninum* infection is usually based
128 on serological techniques, mainly indirect fluorescent antibody tests (IFAT), enzyme-linked
129 immunoassays (ELISA) and agglutination techniques (Huertas-López et al., 2024; Liu et al.,
130 2015; Sinnott et al., 2017; Wyrosdick and Schaefer, 2015). However, these techniques have
131 several limitations that could be especially relevant when used in wildlife, such as the lack of
132 species-specific conjugates (in ELISA and IFAT techniques), the subjective interpretation of the
133 results (in IFAT and agglutination techniques), the labour-intensive nature and the time required
134 to perform the technique (e.g. Western Blot -WB-), the cross-reactivity with other Apicomplexa
135 parasites, as well as the variable sensitivity and specificity depending on the antigen used
136 (Anderson et al., 2007; Dubey and Lindsay, 1996; Dubey et al., 2009; Gondim et al., 2017;
137 González-Barrio et al., 2024; Huertas-López et al., 2022, 2023, 2024; Liu et al., 2015; López
138 Ureña et al., 2023; Sinnott et al., 2017; Wyrosdick and Schaefer, 2015).

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

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

155 ## 2. MATERIAL AND METHODS

156 ### 2.1. Ethics statement

157 This study complied with all Andalusian, Spanish and European legal requirements and
158 guidelines regarding experimentation and animal welfare. Handling procedures and sampling
159 were designed to reduce stress and minimize the impact on the health of the animals. The study
160 was approved by the Ethics on Animal Welfare Committee of the University of Jaén and
161 authorized by the Dirección General de Producción Agrícola y Ganadera (Consejería de
162 Agricultura, Pesca y Medio Ambiente; Junta de Andalucía, Spain).

163 ### 2.2. Study area

164 Located in the centre of the Baetic mountain range in southeastern Spain (37° 12' 40" N, 3° 32'
165 51" O to 36° 56' 26" N, 2° 39' 50" O), the NSSN spreads over 172,318 ha, altitude ranging from
166 300 to 3.482 metres above sea level. The NSSN is formed by the core National Park (85,883 ha)
167 and the surrounding Natural Park (86,355 ha). The climate is generally Mediterranean and cold
168 continental in the highest areas due to the altitude, with seasonal climatic variations (Junta de
169 Andalucía, 2020). The wild ungulate species present in the NSSN are wild boar (*Sus scrofa*), red
170 deer (*Cervus elaphus*) and Iberian ibex, the latter being the most abundant species (Granados et
171 al., 2020).

172

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). Most of the animals were captured within the NSSN boundaries, except for some animals that came from neighbouring mountainous areas and are, therefore, part of the same Iberian ibex metapopulation (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 minutes and stored at -20ºC 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). 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 37ºC 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.

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

217 **3. RESULTS**

218 Seventeen out of the 146 sera (11.6%, central confidence interval 95% 6.4-16.7) were positive
219 according to the *T. gondii* ELISA, while seven of the 147 sera (4.8%, central confidence interval
220 95% 1.3-8.2) yielded positive results in the *N. caninum* ELISA. However, no positive sera were
221 detected by WB for neither *T. gondii* nor *N. caninum* (Supplementary data).

222

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 as assessed by ELISA and WB. 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 13.0% 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 fall within the lower part of the overall range described for all the ruminant species in the Iberian Peninsula (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), whereas for *N. caninum* they cover the whole range previously reported for all the wild ruminant species (0.0% to 12.9%; Almería et al., 2007; Calero-Bernal et al., 2020; García-Bocanegra et al., 2012).

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* antibodies and anti-*N. caninum* antibodies in the domestic goat, using the same native antigens and a monoclonal anti-goat/sheep IgG secondary antibody (Huertas-López et al., 2021; Sánchez-Sánchez et al., 2021). However, and despite the phylogenetic proximity between both species, they are not currently validated for Iberian ibex. Due to the previously mentioned difficulties to validate serological techniques in wild animals, as well as the absence of previous prevalence data

249 that could allow a Bayesian latent class analysis, it is advisable to use a strict positivity criterion,
250 considering only as positive samples those that were positive by at least two techniques (Donahoe
251 et al., 2015). Therefore, we should conclude that none of the sampled animals were seropositive
252 to both parasite infections. In this regard, it should be highlighted that WB is considered a more
253 reliable technique to detect specific antibodies against both parasites (López Ureña et al., 2023;
254 Mazuz et al., 2018); unfortunately, WB is a laborious technique not routinely used in the diagnosis
255 of *T. gondii* and *N. caninum* in wild ungulates (Anderson et al., 2007; Chávez-Velásquez et al.,
256 2004, 2005; Dubey et al., 2009; Olamendi-Portugal et al., 2012). ELISA false positives can occur
257 because of cross reaction with other Apicomplexa species (Huertas-López et al., 2021; Gondim
258 et al., 2017; Nishikawa et al., 2002) or because interferences due to serum quality (Gutiérrez-
259 Expósito et al*.,* 2013). These results reinforce the need to confirm the results obtained by MAT,
260 IFAT and ELISA with other techniques with higher specificity, such as WB, when analysing
261 samples from wild host species where the techniques have not been validated, as the Iberian ibex.
262 The seroprevalences of these parasites described in previous studies in Iberian ibexes from other
263 mountainous areas would probably have been different if the serological results obtained had been
264 confirmed by WB or, at least, by a second technique (Almería et al., 2007, 2018, 2021; Calero-
265 Bernal et al., 2020; García-Bocanegra et al., 2012). Consequently, the values provided for other
266 Iberian populations must be interpreted with caution. Considering that the Iberian ibex usually
267 inhabits mountainous areas (Acevedo and Casinello, 2009), it would be expected that other
268 Iberian populations were seronegative or have low seroprevalences, as in our study. However, we
269 cannot rule out the existence of other area-specific factors that could influence the circulation of
270 both parasites, including ecological, behavioural and management context of the host species.

271 Our study suggests that the habitat occupied by Iberian ibex in SNNS is possibly a hypoendemic
272 area for *T. gondii* and *N. caninum*. However, such eco-epidemiological interpretation could be
273 different if only the results obtained with ELISA techniques were considered. The confirmation
274 by WB that none of the Iberian ibexes studied had specific antibodies to *T. gondii* and *N. caninum*

is consistent with the environmental, ecological and epidemiological characteristics of mountain ecosystem, such as the NSSN. Endemic status of a pathogen in the habitat requires the concurrence of all the eco-epidemiological factors that enable its presence and spread (Webster et al., 2017), including the maintenance of a given host social structure and ecological interactions, the presence of key susceptible species in transmission, as well as the environmental features that ensure the persistence of infective forms in the environment (Cable et al., 2017; Silk and Fefferman, 2021). Specifically, the environmental characteristics of mountainous areas inhabited by the Iberian ibex may influence the infectivity of Apicomplexa oocysts. In particular, prolonged periods of low temperatures in the snowy period, the presence of sparse vegetation cover that favours solar action, as well as the dryness and insolation of rocky areas may be factors that reduce the survival of *T. gondii* and *N. caninum* oocysts (Dubey et al., 2007; Hershey and McGregor, 1987; Shapiro et al., 2019). Beyond such environmental conditions, the presence in mountain ecosystems of definitive hosts for *T. gondii* and *N. caninum* that can spread oocysts is determinant for the maintenance of the sylvatic cycle of these parasites. Specifically, the only *T. gondii* definitive host species present in the NSSN are European wildcat (*Felis silvestris*) and domestic cat (*Felis catus*; Dubey, 2010), and domestic dog for *N. caninum* (Dubey et al., 2007). However, the epidemiological role of the European wildcat in the maintenance of *T. gondii* in the study area is probably minor, since the NSSN is estimated to have 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), so the distinct ecological niches occupied by both host species along the altitudinal gradient minimize their contact, 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, the definitive host for *N. caninum*, is mostly restricted to inhabited areas. Consequently, both domestic species are predominantly linked to humanized areas and not widely distributed throughout the NSSN. So, the environmental conditions for oocyst survival at high altitudes and, notably, the reduced abundance (European wildcat) and restricted distribution (domestic dog and cat) of the oocyst-shedding definitive hosts for *T. gondii* and *N. caninum* probably explain the hypoendemic status and, consequently, the lack of specific antibodies against these two parasites in the population of Iberian ibex in NSSN.

In high mountainous areas inhabited by the Iberian ibex, the epidemiological situation of both *T. gondii* and *N. caninum* may progress towards endemicity, particularly in the presence of a resident population of definitive hosts that continually contaminate the environment with oocysts. In this sense, anthropization is among the main factors that can influence the composition and abundance of host community and, therefore, the epidemiological scenario. In the case of mountain wild ruminants, such as the Iberian ibex in the NSSN, this risk of anthropic origin is related to the increase in the number of dogs and cats associated with human presence (either as visitors or settlers creating new human population nuclei), favouring the environmental propagation of oocysts (Almería et al., 2021; Ballash et al., 2019; Barros et al., 2018; Barroso et al., 2020; Costanzi et al., 2021; Lizana et al., 2021; VanWormer et al., 2013; Wilson et al., 2024). Besides to the anthropogenic influence, mountain ecosystems are particularly vulnerable. In fact, it is predicted that these areas will experience a decrease in available nutrients for herbivores and, consequently, a cascade of ecological effects with results difficult to forecast, including changes in community structure, biotic interactions (Ernakovich et al., 2014; Inouye, 2020) and, specifically, host-parasite interactions (Brooks and Hoberg, 2007; Rohr et al., 2011). These cumulative changes could create new ecological niches suitable for pathogens not previously established or abundant, which may lead to the emergence of refugia for these pathogens (Cohen

et al., 2017; Gsell et al., 2023), such as the disappearance of prolonged periods with snow and freezing temperatures in high mountain areas with longer warm season (Jiménez et al., 2019), allowing the survival of infective stages in the environment (Suh et al., 2024). Simultaneously, the susceptibility of the Iberian ibex and other host species could be affected; in this regard, the "Thermal mismatch hypothesis" suggests that, in general terms, host species become more susceptible to infection as they move away from their own thermal optimum (Cohen et al., 2017).

The potential impact of global change through anthropogenic activity and climate change may significantly shift the ecological and epidemiological dynamics of generalist multi-host parasites such as *T. gondii* and *N. caninum*, threatening the condition of "hypoendemic islands" of mountainous areas, such as the NSSN (Méthot, 2012; Vander Wal et al., 2014). The entry of these parasites in the NSSN could have demographic impacts on the Iberian ibex population, since the lack of previous contact with infective Apicomplexa oocysts could lead to high rates of disease cases and reproductive failure, as it has been observed in other wild ruminants worldwide (Donahoe et al., 2015; Dubey et al., 2007, 2010; Patel et al., 2019; Soler et al., 2022). Moreover, such entry would occur in an epidemiological framework where different pathogens, such as *Sarcoptes scabiei* and *Mycoplasma conjunctivae*, are already endemically present and conditioning the ecology, epidemiology, and demography of the Iberian ibex population in the NSSN (Alasaad et al., 2013; Carvalho et al., 2015; López-Olvera et al., 2015, 2024; Pérez et al., 1997; Sarasa et al., 2011;). Coinfection with different parasites could synergically worsen the health status of the host population, as has been previously demonstrated, increasing the probability of extinction of the host population (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, 2015; 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

354 early-detect and establish fast-response correction and management measures to protect the
355 Iberian ibex population of the NSSN in case of introduction of these two parasites.

356 5. CONCLUSIONS

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

365 CRediT authorship contribution statement

366 **Alejandro Cano-Manuel:** Methodology, Formal analysis, Investigation, Data Curation, Writing
367 - Review & Editing. **José Enrique Granados:** Conceptualization, Investigation, Resources, Data
368 Curation, Writing - Review & Editing, Visualization, Project administration, Funding acquisition.
369 **Gema Álvarez-García:** Conceptualization, Methodology, Resources, Writing - Review &
370 Editing, Supervision, Project administration, Funding acquisition. **Carlos Diezma-Díaz:**
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373 Writing - Review & Editing. **Luis Miguel Ortega-Mora:** Resources, Writing - Review &
374 Editing, Funding acquisition. **Paulino Fandos:** Writing - Review & Editing. **Gregorio**
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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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746

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 %)

747

748 **Figure 1**.

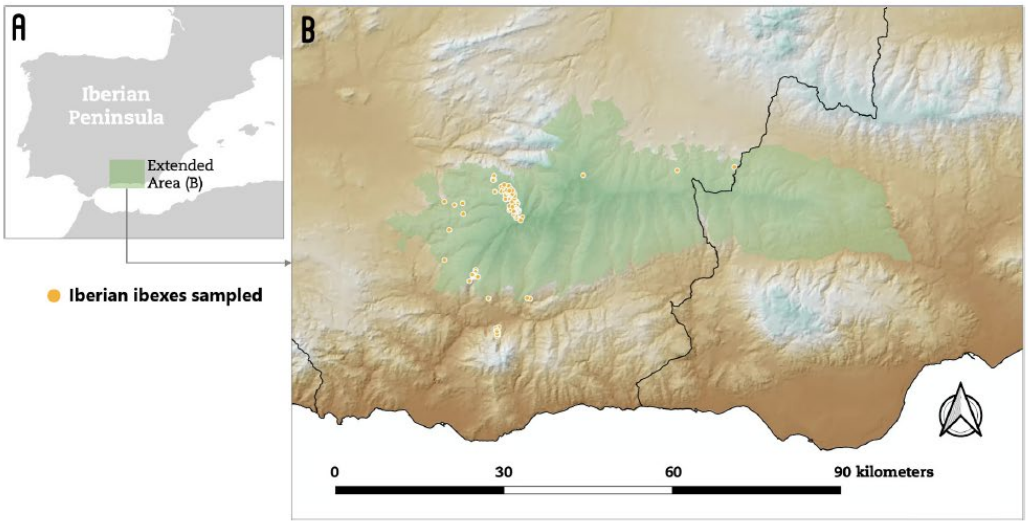

Fig. 1. Location of the captures of the 146 Iberian ibexes sampled (yellow spots on the map). The green area is the Natural Space of Sierra Nevada (NSSN, southeastern Spain). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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