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Accepted 9 June 2004

Abstract

Sera from 456 wild rabbits (Oryctolagus cuniculus) collected between 1992 and 2003 from five geographical regions of Spain were examined for antibodies to Toxoplasma gondii by the modified agglutination test. Antibodies to T. gondii were found in 65 (14.2%) wild rabbits. Prevalence of infection was significantly higher in samples collected from wild rabbits from Catalonia, northeast Spain (53.8%), where rabbits lived in forest, compared to other areas (Huelva and Cádiz, Andalucía, south Spain; Toledo, Castilla-La Mancha, central Spain; and Zaragoza, Aragón, northeast Spain) with more dry conditions, where prevalence ranged from 6.1 to 14.6%. No differences were observed on prevalence and age (young animals < 7 months of age compared to older animals), sex, date of samples collection or season of samples collection. The results indicate that prevalence of T. gondii in some areas of Spain is high, and this finding could have environmental and/or public health implications if wild rabbits are to be used as a source of food.

Keywords: Toxoplasma gondii; Wild rabbits; Oryctolagus cuniculus; Spain; Modified agglutination test

1. Introduction

The European wild rabbit (Oryctolagus cuniculus) is native to the Iberian Peninsula and has been introduced around the world. European rabbit plays a key ecological role in Spanish
Mediterranean ecosystems and their biodiversity (Delibes and Hiraldo, 1981). In addition, it is among the primary species of small game in sport hunting and is a source of food. More than 4 million wild rabbits are hunted in Spain, and they are directly consumed by hunters or derived to food industry (Ministry of Agriculture, 1996).

In Spain, wild rabbit population remains low after having suffered from infectious disease as myxomatosis and rabbit haemorrhagic disease (RHD), both currently endemic diseases in Spain (Calvete et al., 2002). Knowing the prevalence of different pathogens, including parasites, in wildlife species is of major importance in environmental protection strategies (Artois, 1993). The possibility of zoonotic diseases transmitted from wild animals to humans has important public health implications.

Toxoplasma gondii infection is widely prevalent in many species of warm-blooded animals including man (Dubey and Beattie, 1988). Public health concerns associated with T. gondii clearly indicates the need for epidemiological investigation of this infection in animals that can be used as a source of food. Although fatal toxoplasmosis in domestic rabbits (Dubey et al., 1992) and epizootics of toxoplasmosis in wild hares and rabbits mainly from Scandinavia and USA (reviewed by Dubey et al. (1992) and Sedlák et al. (2000)) have been occasionally reported, little is known of the prevalence of antibodies to T. gondii in European wild rabbits. More than 25 years ago, Kapperud (1978) reported T. gondii antibodies in 21% of the 34 wild rabbits from Norway and Sweden and Cox et al. (1981) 7.5% of the 1697 wild rabbits from Victoria, Australia. Recently, Hejlícek et al. (1997), found T. gondii antibodies in 8% of the 79 wild rabbits in Czech Republic. The present study reports the seroprevalence of T. gondii antibodies in 456 wild rabbits from Spain where infection could have ecologic and public health implications.

2. Materials and methods

2.1. Sources of rabbits

Blood samples were collected in five geographical areas that included main areas of wild rabbit population of Spain: Huelva and Cádiz (Andalucía, south Spain), Toledo (Castilla-La Mancha, central Spain), Zaragoza (Aragón, northeast Spain) and areas of Catalonia (northeast Spain). With the exception of Catalonia, where rabbits were hunted in forest areas, the habitat of the rabbits living in the other areas consisted mainly of low hillocks interspersed with small field of wheat and barley. The main natural vegetation was sparse steppe scrub which was restricted to the hillocks.

Blood samples were collected from an incision in the auricular marginal vein from 412 wild rabbits captured from 4 of the 5 geographical areas studied. Rabbits were sexed and classified as young (up to 7 months) or adults (over 7 months) by the external assessment of the degree of ossification of the tibial and distal ulnar epiphyses (Watson and Tyndale-Biscoe, 1953). In those areas, the animals were released after sampling. In Catalonia, rabbits were hunted and blood collected immediately afterwards, no age or sex data were available from hunted rabbits in this area. Blood samples were centrifuged and sera stored at −20 °C until analysis was performed.
2.2. Serological examination for *T. gondii*

Sera were analyzed for antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Sera were diluted at 1:25, 1:50, and 1:500 and specimens with doubtful results were reexamined. Positive and negative controls were included in each test. Although the specificity and sensitivity of MAT have not been evaluated for the diagnosis of toxoplasmosis in wild rabbits, it is the most evaluated and specific test for the diagnosis of toxoplasmosis in animals, particularly pigs (Dubey et al., 1995; Dubey, 1997). A titer of $\geq 1:25$ was considered indicative of *T. gondii* infection in wild rabbits as has been for other species (Dubey and Beattie, 1988).

2.3. Statistical analysis

Seroprevalence was statistically analyzed considering the variables geographical area, date of sample collection, season of sample, sex and age of the animals. The statistical data analysis were performed using the SPSS 11.0 Statistical Program by $\chi^2$ test. The differences between variables were analyzed by Bonferroni or Tukey–Kramer tests. For multiple comparison, Dunn’s test was performed and when variances were not homogenous, non-parametric test was performed. The differences were considered statistically significant when $P \leq 0.05$.

3. Results

Antibodies (MAT $\geq 1:25$) to *T. gondii* were found in 65 of the 456 (14.2%) wild rabbits with titers of 1:25 in 34 (52.3%), 1:50 in 26 (40.0%), and $\geq 1:500$ in 5 (7.7%) wild rabbits.

No statistically significant differences were observed in the prevalence of infection and sex or age of the animals (Table 1). Antibodies were found in 11.6% of the 164 males and 12.6% of the 222 females ($P = 0.36$); in 40 of the 363 (11.0%) adult ($>7$ months) wild rabbits and in 11 of the 67 (16.4%) young ($\leq 7$-month-old) ($P = 0.15$).

Rabbits from Catalonia had the highest (53.8%) prevalence of *T. gondii* antibodies, being significantly higher than prevalence of antibodies in the other areas, where prevalence ranged from 6.1 to 14.6% ($P < 0.001$) (Table 1).

Not statistically significant differences were observed between prevalence of infection and season of collection (March–September versus October–February) or date of collection (recent samples collected from 2002 onwards versus samples collected before 2002, Table 1).

4. Discussion

In the present study, the 14.2% seroprevalence of *T. gondii* in wild rabbits was not related to age, sex or season. The main factor affecting prevalence of infection was location. A significantly higher ($P < 0.001$) prevalence of infection was observed in Catalonia, where rabbits were hunted in forest areas, compared to rabbits from other areas of Spain captured.
in mostly dry areas. One explanation could be that while alive capture should not select weak animals, hunting could have had a more restricted selection of debilitated animals by sickness, in this case related to toxoplasmosis. Another more probable possibility explaining the higher prevalence in Catalonia, was the habitat in which the animals lived. Cox et al. (1981) and Hejlíček et al. (1997) found considerable variation between localities and strongly positive sera came from rabbits collected in a small number of localized areas. Similarly, T. gondii prevalence in wild mammals in the United States showed that similar biotypes or soil types might result in similar prevalence among animals from different areas (Smith and Frenkel, 1995). Areas of deciduous woodland and forest canopies, resulting in shade and relatively higher humidity, had the highest prevalence of infection, while areas characterized by less shade, less rain and higher rates of evaporation and therefore more dessication had similar lower prevalence rates (Smith and Frenkel, 1995). In the present study, similar lower prevalence of infection were observed in different areas of Spain with mostly dry habitats while prevalence in the more humid forest areas of Catalonia was the highest.

Rabbits probably become infected by T. gondii ingesting food or water contaminated with T. gondii oocysts excreted by felids. Congenital transmission in rabbits is possible (Uhlíková and Hübner, 1973), but its frequency in wild mammals is unknown.

Urban cats from Barcelona (Catalonia) had high prevalence of T. gondii antibodies (45% of 220), with the highest prevalence of infection in feral cats (Gauss et al., 2003). The high prevalence of cats in Catalonia indicate also high possibility of oocyst excretion to surrounding areas, may be affecting the wild rabbit prevalence in this area.

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**Table 1**

Prevalence of *T. gondii* in wild rabbits from Spain

<table>
<thead>
<tr>
<th>Category</th>
<th>No. examined</th>
<th>No. positive a</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults (&gt;7 months) a</td>
<td>363</td>
<td>40</td>
<td>11.0</td>
</tr>
<tr>
<td>Juvenile (&lt;7 months) a</td>
<td>67</td>
<td>11</td>
<td>16.4</td>
</tr>
<tr>
<td><strong>Location (habitat)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huelva (south-1) a</td>
<td>93</td>
<td>16</td>
<td>17.2</td>
</tr>
<tr>
<td>Cadiz (south-2) a</td>
<td>148</td>
<td>9</td>
<td>6.1</td>
</tr>
<tr>
<td>Toledo (central) a</td>
<td>59</td>
<td>7</td>
<td>11.9</td>
</tr>
<tr>
<td>Zaragoza (northeast-1) a</td>
<td>130</td>
<td>19</td>
<td>14.6</td>
</tr>
<tr>
<td>Catalonia (northeast-2) b</td>
<td>26</td>
<td>14</td>
<td>53.8</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male a</td>
<td>183</td>
<td>20</td>
<td>10.9</td>
</tr>
<tr>
<td>Female a</td>
<td>247</td>
<td>31</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Season</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March–September a</td>
<td>139</td>
<td>20</td>
<td>14.4</td>
</tr>
<tr>
<td>October–February a</td>
<td>317</td>
<td>45</td>
<td>14.2</td>
</tr>
<tr>
<td><strong>Date</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before 2002 a</td>
<td>282</td>
<td>42</td>
<td>14.9</td>
</tr>
<tr>
<td>From 2002 (included) a</td>
<td>174</td>
<td>23</td>
<td>13.2</td>
</tr>
</tbody>
</table>

a Statistically significant differences when different letters (a, b) in columns within category.

b MAT ≥ 1:25.
Rabbits are common prey for many carnivorous species with animals debilitated by any cause being easy prey (Temple, 1987; Trout et al., 1992). In Spain, rabbits are mainly preyed by red foxes (Vulpes vulpes), one of the most abundant wild carnivore in Spain, but also by the Iberian lynx (Lynx pardinus). The Iberian lynx is one of the most endangered felid species in the world, it is only present in a few locations and mainly feed on wild rabbits (Delibes, 1980; Palma et al., 1999). Therefore, *T. gondii* infection in rabbits can be an important source of *T. gondii* infection in Iberian lynx or foxes in Spain, and therefore could concern wildlife conservation (Delibes, pers. commun.). Although the *L. pardinus* has not been examined for *T. gondii* oocyst shedding, it is a fair assumption that they excrete *T. gondii* oocysts as do all other felids tested (Dubey and Beattie, 1988).

Although *T. gondii* infection can be fatal in domestic rabbits, it probably causes no harm to most rabbits. Beverley et al. (1954) found dye test antibodies in 34% of the 321 wild rabbits in UK. More importantly, they found that the prevalence of *T. gondii* antibodies was more than twice in persons who handled rabbits and even higher in rabbits trappers (Beverley et al., 1954). Hejlícek and Literák (1994) isolated *T. gondii* from 54 of the 304 (17.8%) rabbits in South Bohemia. Thus, latently infected rabbits can harbor viable *T. gondii*.

Acknowledgements

We would like to thank Rosa Estrada for helpful assistance in this work.

References


