



## Short communication

Seroprevalence of *Toxoplasma gondii* in North-eastern Atlantic harbor seal (*Phoca vitulina vitulina*) and grey seal (*Halichoerus grypus*)O. Cabezón<sup>a</sup>, A.J. Hall<sup>b</sup>, C. Vincent<sup>c</sup>, M. Pabón<sup>d</sup>, I. García-Bocanegra<sup>e</sup>, J.P. Dubey<sup>f</sup>, S. Almería<sup>d,g,\*</sup><sup>a</sup> Servei d'Ecopatologia de Fauna Salvatge, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain<sup>b</sup> Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, St Andrews, UK<sup>c</sup> Littoral Environnement Sociétés, Université de La Rochelle, 17 000 La Rochelle, France<sup>d</sup> Departament de Sanitat i d'Anatomia Animal, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain<sup>e</sup> Departamento de Sanidad Animal, Facultad de Veterinaria, UCO, Campus Universitario de Rabanales, 14071 Córdoba, Spain<sup>f</sup> Animal Parasitic Diseases Laboratory, Building 1001, Animal and Natural Resources Institute, Agriculture Research Service, United States Department of Agriculture, Beltsville, MD 20705-2350, USA<sup>g</sup> Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

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## ABSTRACT

Antibodies to *Toxoplasma gondii* were determined in serum samples from 47 grey seals (*Halichoerus grypus*) and 56 harbor seals (*Phoca vitulina vitulina*) from the Atlantic coasts of United Kingdom and France. Antibodies to *T. gondii* assayed by the modified agglutination test (MAT) were found in 14 (13.6%; IC<sub>95%</sub>: 7.0–20.2) of 103 seals tested, with titres of 1:25 in 13 seals and 1:50 in 1 seal. Seroprevalence against *T. gondii* (MAT 1:25 or higher) was significantly higher in grey seals (23.4%) compared to harbor seals (5.4%). No significant differences were found between seroprevalence against *T. gondii* and sex, age or geographical locations. These results show natural exposure of European harbor and grey seals to *T. gondii* oocysts in the Atlantic Ocean. To the best of our knowledge, this is the first serological survey of *T. gondii* in European grey and harbor seals.

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## 1. Introduction

*Toxoplasma gondii* is an intracellular apicomplexan protozoan of worldwide distribution. It causes important clinical disease in many species of animals, including humans (Dubey, 2009). *T. gondii* can infect many species of domestic and wild mammals, with felids as definitive hosts (reviewed by Dubey, 2009). *T. gondii* is recognized as an important pathogen in marine mammals (Dubey, 2009). *T. gondii* infection is considered to be a major cause of mortality in sea otters (*Enhydra lutris*) (Cole et al., 2000; Kreuder et al., 2003; Thomas et al., 2007) and fatal toxoplasmo-

sis has been diagnosed in the Pacific harbor seal (*Phoca vitulina richardsi*) (Van Pelt and Dieterich, 1973; Miller et al., 2001). Numerous serologic surveys to determine the seroprevalence of antibodies against *T. gondii* have been carried out in seals (Fam. Phocidae) and *T. gondii* antibodies have been found in several species such as Pacific harbor seal (Lambourn et al., 2001; Dubey et al., 2003), western Atlantic harbor seal (*Phoca vitulina concolor*) (Measures et al., 2004), kuril harbor seal (*Phoca vitulina stejnegeri*) (Fujii et al., 2007), ringed seal (*Phoca hispida*), bearded seal (*Erignathus barbatus*) and spotted seal (*Phoca largha*) (Dubey et al., 2003), grey seal (*Halichoerus grypus*) and hooded seal (*Cystophora cristata*) (Measures et al., 2004), and worldwide reports in these animals were recently reviewed (Dubey, 2009). Compared to these reports, antibodies against *T. gondii* were not found in harp seal (*Phoca groenlandica*), ringed seal (*P. hispida*) and hooded seal (*C.*

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*cristata*) in the Northeast Atlantic Ocean (Oksanen et al., 1998). To our knowledge, no serologic studies have been performed in eastern-Atlantic harbor seal (*Phoca vitulina vitulina*) and European grey seal (*H. grypus*) populations. These species are resident year-round, and are generally coastal (harbor seals) or slightly offshore (grey seals) in foraging behaviour, both frequenting and hauling out inshore.

Harbor seal (*P. v. vitulina*) and grey seal (*H. grypus*) are listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2010) as “least concern”. However, grey seal populations are still below the estimated populations one hundred years ago in the North-eastern Atlantic (Harding and Härkönen, 1999) and harbor seal population dynamics within regional subpopulations vary dramatically (Härkönen et al., 2006). In 1988 more than 20,000 harbor seals were estimated to have died from a phocine distemper virus (morbillivirus) epidemic in European waters (Dietz et al., 1989; Reijnders, 1989). A similar outbreak in 2002 killed approximately 30,000 harbor seal individuals (Härkönen et al., 2006). Information about seroprevalence of *T. gondii*, which could be cause of disease in marine mammals, is therefore of importance for the conservation and management of these seal species.

The objective of this study was to determine the seroprevalence of *T. gondii* in North-eastern Atlantic populations of grey and harbor seals inhabiting the North Sea and adjacent waters, two of the most common *Phocidae* species in all the North Atlantic Ocean.

## 2. Material and methods

Sera from grey seals ( $n=47$ ) and harbor seals ( $n=56$ ) were collected from animals captured live and released from Atlantic coasts of Scotland and France between 1998 and 2004. Grey seals sampled around the coast of UK were from Abertay sands ( $n=5$ ) ( $56^{\circ} 44'N$ ,  $-02^{\circ} 76'E$ ), the Farne Islands ( $n=8$ ) ( $55^{\circ} 63'N$ ,  $-01^{\circ} 63'E$ ) and Wales & Dee estuary ( $n=19$ ) ( $53^{\circ} 32'N$ ,  $-03^{\circ} 15'E$ ) and the Molene population ( $n=15$ ) ( $48^{\circ} 78'N$ ,  $-03^{\circ} 61'E$ ) from Northwest France. The harbor seals sampled were all from UK: St. Andrews Bay ( $n=18$ ) ( $56^{\circ} 35'N$ ,  $-02^{\circ} 82'E$ ), the Moray Firth ( $n=15$ ) ( $57^{\circ} 65'N$ ,  $-04^{\circ} 03'E$ ), Orkney ( $n=18$ ) ( $58^{\circ} 96'N$ ,  $-02^{\circ} 95'E$ ), and Islay and Jura ( $n=5$ ) ( $55^{\circ} 89'N$ ,  $-05^{\circ} 89'E$ ) from Great Britain. Data were collected regarding sex of each animal, age of the animals, geographic area and year of sampling. Age of the animals was recorded in relation of their weight and length (Thompson and Härkönen, 2008a,b). All animals were sampled under UK Home Office Project and Personal Licences as issued to the Sea Mammal Research Unit under the UK Animal (Scientific Procedures) Act, 1986.

Blood was collected from the extradural vein using the Vacutainer system (Becton Dickinson, Oxford, UK) into serum collection tubes and centrifuged at 1200 rpm for 15 min. Separated sera were then stored at  $-20^{\circ}C$  until analyzed.

Separated sera were examined by the modified agglutination test (MAT) to detect antibodies against *T. gondii* as described previously (Dubey and Desmonts, 1987). Sera were tested at 1:25, 1:50, 1:100 and 1:500 dilutions. Commercial positive control (Toxotrol-A, Biomérieux, France) diluted from 1:25 to 1:3200 (with a minimum titre of

**Table 1**

Seroprevalence of *T. gondii* in North-eastern Atlantic harbor seal (*Phoca vitulina vitulina*) and grey seal (*Halichoerus grypus*) populations.

Species	Area of study	<i>T. gondii</i> (MAT)
Grey seal ( <i>H. grypus</i> )	Abertay Sands	60.00% (3/5)
	Molene	26.67% (4/15)
	Farne Islands	12.50% (1/8)
	Wales & Dee estuary	15.79% (3/19)
	Total	23.40% (11/47)
Harbor seal ( <i>P. vitulina</i> )	St. Andrews bay	5.56% (1/18)
	Moray Firth	6.67% (1/15)
	Orkney	5.56% (1/18)
	Islay and Jura	0.00% (0/5)
	Total	5.35% (3/56)

1:200) was included in each test. Negative controls were also included in all tests. Titres of 1:25 or higher were considered positive and those with equivocal results were re-examined. The utility of this method in seals has been demonstrated with animals experimentally infected with *T. gondii* (Gajadhar et al., 2004).

Data were collected regarding sex and age of each animal (adults, sub-adults or pups), geographic area and year of sampling. No age data were recorded for 6 seals. Associations between serological results and independent variables such as species, sex, age, geographic area and year of sampling were analyzed using a Pearson's chi-square test. When observations per category were less than six, Fisher's exact test was used. Differences between variables were analyzed by Bonferroni or Tukey tests. Differences were considered statistically significant when  $P$ -value  $< 0.05$ . Statistical analyses were performed using SPSS 15.0 (Statistical Package for Social Sciences (SPSS) Inc., Chicago, IL, USA).

## 3. Results

Antibodies (MAT  $\geq 1:25$ ) against *T. gondii* were found in 14 (13.6%; IC<sub>95%</sub>: 7.0–20.2) of 103 seals tested, with titres of 1:25 in 13 seals (92.9%) and 1:50 in 1 seal (7.1%). Significantly higher seroprevalence ( $P=0.01$ ) was observed in grey seals (11 of 47, 23.4%) compared to harbor seals (3 of 56, 5.4%) (Table 1). No statistically significant differences were found between prevalence of infection and sex (10 of 47 females, 21.3%, and 4 of 56 males, 7.1%), age (7 of 43 adults, 16.3%, 5 of 40 sub-adults, 12.5% and 1 of 12 pups, 7.1%) and geographical location (Table 1). Seals with positive antibody titres against *T. gondii* were detected in all the studied areas with exception of harbor seals from Islay and Jura (West coast of Scotland). Although no statistically significant differences were observed among years of sampling, it was interesting to observe an increased seroprevalence in grey seals from Molene sampled in 2002 (4 of 9 sampled, 44.4%) compared to those sampled in 1999 (0 of 6 sampled, 0%) ( $P=0.1$ ).

## 4. Discussion

Seroprevalence studies of *T. gondii* antibodies in pinnipeds have been reported in several species but in a few restricted geographical areas: North America, Hawaii

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