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Short communication

Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption

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ABSTRACT

Prevalence of antibodies to *Toxoplasma gondii* and risk factors associated with infection were assessed in food animals from the North of Portugal for human consumption. Antibodies were assayed by means of the modified agglutination test with a cut-off titre of 100 for cattle, and 20 for sheep, goats, and pigs; 7.5% of 161 cattle, 33.6% of 119 sheep, 18.5% of 184 goats, and 9.8% of 254 pigs were seropositive. Among the risk factors examined animal age was an important risk factor for seropositivity to *T. gondii*. The consumption of raw or undercooked meat should be regarded as an important source of infection to people in the study area.

1. Introduction

Toxoplasmosis is a worldwide zoonosis. The consumption of undercooked meat infected with *Toxoplasma gondii* is a major risk factor of infection in humans, especially in Europe, and several outbreaks were epidemiologically linked to ingestion of infected meat (Cook et al., 2000; Dubey, 2010). Among the food animals, *T. gondii* infections are more prevalent in pigs, sheep, and goats than in cattle. Worldwide prevalence of *T. gondii* in food animals was summarized previously (Dubey and Beattie, 1988; Dubey, 2010). However, data are limited from Portugal (Esteves, 2006; de Sousa et al., 2006; Valadas et al., 2006; Sousa et al., 2009). Here, we report seroprevalence and associated risk factors for *T. gondii* infection in cattle, sheep, goats, and pigs from the North of Portugal for human consumption.

2. Materials and methods

2.1. Animals and samples

Between March 2008 and March 2010, blood samples were obtained from 161 cattle, 109 sheep, 54 goats, and 254 pigs slaughtered in abattoirs from the North of Portugal. Sera were additionally obtained from 10 sheep and 130

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goats in farms from the same region. All animals had been born and raised in the North of Portugal and were intended for human consumption. Sera were separated by centrifugation and stored at −20 °C until serological testing.

Cattle were from 107 sources, and their defined breeds comprised Frisian and Portuguese autochthonous beef cattle breeds (Arouquesa, Maronesa and Mirandesa); crossbreeds included crosses of Belgian Blue, Charolais and Limousin. The median age of cattle was 8.0 months (interquartile range [IQR]: 8.0–12.0). Three age groups were established: calves (≤8 months), bullocks/heifers (9–12 months) and adult animals (≥13 months).

Sheep were from 12 flocks, and defined-breed sheep included autochthonous Churra Badana and Churra da Terra Quente. Crosses of Churra and Merino comprised the crossbreed group. Three age groups were established: one for suckling lambs (≤6 months), one for grazing lambs (7–18 months) and another one for adult sheep (≥19 months). Goats were from 14 sources and were raised in an extensive husbandry system.

Pigs were from 14 farms, and their defined breeds included Bísaro (autochthonous), Large White and Piétrain. The crossbred group comprised crosses from Bísaro pigs. Animals were grouped as piglets (≤3 months), fattening pigs (4–9 months) and breeding pigs (≥10 months).

Additional data collected for each sampled animal included gender, breed and/or husbandry system (intensive, semi-intensive or extensive).

2.2. Serologic testing

Serum samples were tested for immunoglobulin G antibodies to *T. gondii* by the modified agglutination test (MAT), using a commercial kit (Toxo-Screen DA®, bioMérieux, Lyon, France). Sheep, goat and pig sera were diluted at 1:20, 1:400, 1:1600 and 1:6400, and cattle sera at 1:100, 1:400, 1:1600 and 1:6400. Positive and negative controls supplied with the kit were included in each testing plate. Results obtained with the MAT were expressed as an antibody titre, i.e. the reciprocal of the highest dilution at which agglutination (at least one half of the well's diameter) was still visible after 5–18 h incubation at room temperature. Cut-off titres of 20 for sheep, goats (Sousa et al., 2009) and pigs (Dubey et al., 1995) and of 100 for cattle (Dubey and Jones, 2008) were chosen to maximize both sensitivity and specificity of the test.

2.3. Data analysis

Assuming a default 50% seroprevalence value, a 95% confidence level and a 10% absolute error, at least 97 animals from each species were calculated to include in this study (Thrusfield, 2005). The chi-square or Fisher's exact test were used to compare seroprevalence values among and between animal species. Independent variables with a significant difference between groups (probability [p] value <0.05) were selected for multiple logistic regression analysis to identify independent risk factors for seroprevalence, calculating odds ratios (OR) and their 95% CI (Altman, 1991). Statistical analyses were done with SPSS 11.5 software for Windows.

3. Results and discussion

Antibodies to *T. gondii* (only titre of 100) were detected in 12 (7.5%) of the 161 cattle. Antibodies were detected in 33.6% of sheep with titres of 16 in 20, 400 in one, 1600 in three and ≥6400 in 20; 18.5% of goats were seropositive with titres of 20 in 25, 400 in one, 1600 in four, and ≥6400 in four. Thus, many sheep had high titres. Antibodies were found in and 9.8% of pigs, with titres of 20 in 16, 400 in eight, and 1600 in one. Age was identified as a risk factor for seropositivity to *T. gondii* in sheep, goats, and pigs (Table 1).

This is the first epidemiological study on *T. gondii* infection in goats from Portugal as well as in cattle from the North region of the country. Also in northern Portugal, by using the same serological test (MAT), seroprevalence values were 35.8% in domestic cats (Lopes et al., 2008) and 38.0% in dogs (Lopes et al., 2011b). In North and Central Portugal 61.1% of wild animals were seropositive to *T. gondii* (Lopes et al., 2011c). These results support the scenario of a considerable presence of sporulated oocysts, as well as infected intermediate hosts, in the local environment. In addition, and also in northern Portugal, the overall seroprevalence of *T. gondii* infection in women of childbearing age was 24.4% (Lopes et al., 2011a). Other recent studies have found a seroprevalence of 6.2% in sheep from southern Portugal (Esteves, 2006) and 17.1% in sheep from the northeast (Sousa et al., 2009). Seropositivity in pigs from Portugal was reported to be 5.2% (Valadas et al., 2006) or 8% (Esteves, 2006) from the south and 15.6% from northeastern part of Portugal (de Sousa et al., 2006); the later authors isolated viable *T. gondii* from 15 of 37 seropositive pigs. There are no reports of isolation of viable *T. gondii* from sheep and goats from Portugal.

The present serological survey was based mostly on samples collected from abattoirs and does not represent a national or regional population prevalence. Nevertheless, the data are important as a starting point to investigate regional epidemiology of toxoplasmosis in food animals for human consumption.

Serologic prevalence of *T. gondii* varies with the serologic test, and cut-off values. In the present study we used the MAT. Currently, the MAT is considered the most reliable, sensitive and specific test for the detection of antibodies to *T. gondii* in various hosts and it does not require species-specific reagents (Desmonts and Remington, 1980; Dubey and Desmonts, 1987). The MAT has been verified in domestic and feral pigs (Dubey et al., 1995; Richomme et al., 2009), sheep (Dubey et al., 2008; Halos et al., 2010), and goats (Dubey et al., 2011) using isolation of *T. gondii* as the standard (Dubey, 2010). None of the serologic tests have been verified in cattle naturally exposed to *T. gondii* infection because the parasite was rarely isolated from tissues of naturally infected cattle. One cow from which viable *T. gondii* was isolated in USA had a persistent MAT titre of 1000 or higher (Dubey, 1992). Based on studies in experimentally infected cattle a titre of 100 was suggested as a screening dilution for the detection of *T. gondii* antibodies in cattle (Dubey et al., 1985). In the present study all seropositive animals had a titre of only 100. We are therefore uncertain about the significance of the findings. Opsteegh et al. (2011) evaluated ELISA and MAT in naturally exposed