COMPARATIVE CROSS-SECTIONAL STUDY OF *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII*: SEROPREVALENCE IN SHEEP OF GREECE AND NORTH-EASTERN SPAIN

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Abstract

A cross-sectional study was carried out to compare and to analyse the seroprevalence of Neospora caninum and Toxoplasma gondii between Greece and Catalonia (NE Spain) and to analyse the relationship between the prevalence of infections and the origin of sheep. Serum samples of 80 sheep from farms in Greece, 10 sheep from Romania slaughtered in Greece, and 90 sheep from farms in Spain were collected. Antibodies against T. gondii were detected in 88 out of 180 (48.9%) (95% confidence interval (CI) = 41.4%-56.4%) sera analyzed, while only 2 (1.2%; CI= 0.1%-4.0%) serum samples were positive for antibodies against N. caninum. T. gondii seroprevalence showed similar levels in the countries sampled, and no statistically significant differences were observed among countries; whereas N. caninum was only detected in samples from Greece (2 out of 80; 2.5%). Furthermore, there was a high variability in the infection levels of T. gondii among regions of origin, and the

region of sheep origin was the only significant risk factor associated with T. gondii seroprevalence. In conclusion, contact with T. gondii is common in sheep unlike N. caninum in the analyzed areas.

Keywords: *infection levels; risk factor; cross sectional study; Catalonia; Romania*

Introduction

Neospora caninum and *Toxoplasma gondii* are two closely related intracellular protozoan parasites with a worldwide distribution. *Toxoplasma gondii* can infect all warm-blooded species as intermediary hosts and felids as definitive hosts (reviewed by Dubey, 2009). *Neospora caninum* infects several domestic and wild mammal species (Dubey *et al*., 2007; Dubey and Schares 2011, Almería, 2013) with dogs and several species of wild canids (coyotes, dingoes and wolves) confirmed as definitive hosts to date (reviewed by Almeria, 2013). Both parasites cause reproductive problems in their intermediate hosts, including sheep. In pregnant sheep toxoplasmosis may cause foetal death and resorption, abortion, foetal mummification, stillbirth and neonatal death (Dubey and Lindsay 2006; Buxton and Rodger, 2007) while neosporosis produces abortion (Moreno *et al.*, 2012). These protozoan parasites are of great economic importance to the sheep industry, of which a total number of 68.1 million breeding ewes is reported in the European Union (Hybu Cig Cymru- Meat Promotion Wales: The Current trends in breeding ewe numbers and meat production within the EU: https://goo.gl/J7z6EH).

Several epidemiological studies have recently investigated the seroprevalence of these two parasites in sheep in several countries (García-Bocanegra *et al*., 2013; Lopes *et al.*, 2013; Hecker *et al*., 2013; Langoni *et al.*, 2011; Panadero *et al*., 2010; Bártová *et al.*, 2009) but none in a comparative way between countries. The objective of the present cross-sectional study was i) to compare and to analyse the seroprevalence of *N. caninum* and *T. gondii* between Greece and Catalonia (NE Spain) and ii) to analyse the relationship between the prevalence of infections and the origin of sheep.

Materials and Methods

Serum samples of 180 sheep from Greece and Spain were collected. In Greece, 80 clinically healthy and randomly selected sheep were sampled in farms whose owners agreed to participate in the study and from 10 sheep in an abattoir. Because sheep sampled in abattoir could originate from Greece and other countries, their origin was based on the accompanying official documents for each animal. In the present study the 10 sheep sampled in an abattoir originated from Romania. In Spain, serum samples (n=90) from sheep were supplied by the Animal Health Laboratory of the Catalan Government (NE Spain). All blood samples were centrifuged, and the obtained sera stored at −20 °C until analysed.

A commercially available indirect ELISA kit (Chekit *Neospora caninum* Antibody ELISA test kit, IDEXX Laboratories, Berne, Switzerland) that detects antibodies against *N. caninum* in samples from ruminants including sheep was used following the manufacturer's instructions. The assay uses a *N. caninum* antigen and an anti-ruminant IgG conjugate, which detects bovine, ovine, and caprine sera.

Antibodies against *T. gondii* were assayed by the modified agglutination test (MAT) described by Dubey and Desmonts (1987). Sera were diluted two fold starting at 1:25 to 1:500. A positive and a negative control were included in the test. Titers of 1:25 or higher were considered positive as in previous studies in ruminant species, including sheep (Lopes et al., 2013).

The relationship between the prevalence of infections and the investigated risk factors (country of sheep, geographic region within country, and *N. caninum*-positive farms) was analyzed by defining a binary outcome variable where positive samples were coded as 1, while negative samples were coded as 0. The Chi-square or alternatively the Fisher's exact tests were used to analyze the association of all examined factors as independent categorical variables with *T. gondii* prevalences. Only the multilevel variable which was significant at $p<0.05$ and was subsequently used in a univariate logistic regression model. Overall fit of the logistic regression model was assessed using the Hosmer–Lemeshow goodness-of-fit statistics. Results are presented as odds ratios (OR) with 95% confidence intervals (95% CI). In all cases, the statistical significance was considered at 5% level. Missing observations were excluded from the analysis. No statistics was applied to *N. caninum* prevalences due to the very low number of positives cases (2/180). All statistics were performed using the SPSS version 13.0 statistical package.

Results

Antibodies against *T. gondii* were detected in 88 out of 180 (48.9%) [95% confidence interval (CI) = 41.4%-56.4%] sera analyzed, while only 2 (1.2%; CI= 0.1%-4.0%) serum samples were positive for antibodies against *N. caninum*. *T. gondii* seroprevalence showed similar levels in the countries sampled, and no statistically significant differences were observed among countries (Table 1), whereas *N. caninum* was only detected in samples from Greece (2 of 80; 2.5%). The region of sheep origin was the only significant risk factor associated with *T. gondii* seroprevalence (Table 1). There was a high variability in the infection levels among the regions of animal origin: Trikala was the region with the highest prevalence (90%), and Loutraki-Corinthia was the region with the lowest prevalence (10%); the risk of infection was at least 32-fold higher for the regions of Trikala, Baix Empordà, Asimenio-Didimotiho, Xilokeriza-Corinthia Velestino-Volos, Giannitsa, Gironès and Baix Llobregat compared with Loutraki-Corinthia ($p<0.05$). The levels of seroprevalence in Bages, Alt. Urgell, Selva, Pallars. Sobirà, Osona, Aliveri-Evia Alt Empordà, Priorat and Sitihori-Didimotiho were not significantly different from Loutraki-Corinthia level (Table 1).

Table 1 Univariate analysis of factors associated with Toxoplasma infections in sheep. Results are presented as odd ratios (OR) and 95 % confidence intervals (CI).

Factor	N[a]	n[b]	Prevalence (%)	OR	95% CI	p-value
Country						0.20
Greece	80	45	56.3	1.28	0.34-4.7	0.70
Catalonia-Spain	90	38	42.5	0.73	0.19-2.7	0.63
Romania	10	5	50	1.00		
Region						0.00*
Trikala	10	9	90	81.00	4.36-1504.46	0.00*
Baix Empordà	9	8	89	72.00	3.84-1349.54	0.00*
Asimenio-Didimotiho	10	8	80	36.00	2.72-476.27	0.00*
Xilokeriza-Corinthia	10	8	80	36.00	2.72-476.27	0.00*
Velestino-Volos	10	8	80	36.00	2.72-476.27	0.00*
Giannitsa	10	8	80	36.00	2.72-476.27	0.00*
Gironès	9	7	77.8	31.50	2.35-422.29	0.00*
Baix Llobregat	9	7	77.8	31.50	2.35-422.29	0.00*
Bages	9	5	55.6	11.25	0.97-130.22	0.05
Alt Urgell	9	3	33.3	4.50	0.37-54.15	0.23
Selva	9	2	22.2	2.57	0.19-34.47	0.47
Pallars Sobirà	9	2	22.2	2.57	0.19-34.47	0.47
Osona	9	2	22.2	2.57	0.19-34.47	0.47
Aliveri-Evia	10	2	20	2.25	0.17-29.76	0.53
Alt Empordà	9	1	11.1	1.12	0.06-21.08	0.93
Priorat	9	1	11.1	1.12	0.06-21.08	0.93
Sitihori-Didimotiho	10	1	10	1.00	0.05-18.57	1.00
Loutraki-Corinthia	10	1	10	1.00		
Unknown	10	5	50			
***Neospora* positivity**						0.23
Negative	178	86	48.3	-	-	-
Positive	2	2	100	-	-	-

[a] Total number of samples examined
[b] Total number of positive samples
CI: Confidence interval

Discussion

Previous studies have reported a wide distribution of *T. gondii* in mammals and high seroprevalence levels in small ruminants, particularly sheep, in which *T. gondii* infection is one of the main causes of related abortion worldwide (reviewed Dubey, 2009). The present preliminary study showed similar *T. gondii* seroprevalence levels in Greece and Spain, as well as in those few animals from Romania that were slaughtered in Greece. These results are in agreement with the seroprevalence levels observed in recent studies of *T. gondii* in sheep in Mediterranean countries, including those from the Iberian Peninsula and Greece: (48.6% in Greece, (Tzanidakis *et al*., 2012); 33.6% in Portugal (Lopes *et al*., 2013), and 49.3% in Southern Spain (García-Bocanegra, *et al*., 2013). The reason for this uniformity is probably related to the similar climate conditions (Samra et al., 2007), since the analyzed countries are located within the warm temperate and subtropical climate zones (Peel *et al*., 2007).

Although the mean seroprevalence levels in the countries were similar, significant differences were observed among regions within countries, which was the only significant factor associated with *T. gondii* in the present study and in agreement with the results from other studies (da Silva et al., 2012). Although the samples in the present study did not represent the national population, the study should be considered as a starting point to carry out regional epidemiological studies of *T. gondii*. Nevertheless, the noticeable seroprevalence level of *T. gondii* in samples from slaughtered animals is of interest from a health point of view since it is indicative of infection in animals intended for human consumption.

Another interesting observation in the present study was the relatively low prevalence of *N. caninum* compared to *T. gondii* in sheep. A similar observation was also detected in previous studies in sheep where the prevalence of *N. caninum* was lower than the prevalence of *T. gondii* (Rossi *et al.*, 2011; Spilovská et al., 2009; Panadero et al., 2010; Bártová et al., 2009; Soares et al., 2009; Ueno et al., 2009). The trend of higher susceptibility of sheep to *T. gondii* is in the opposite direction to the trend of lower susceptibility of cattle to *T. gondii* compared to *N. caninum*. Even though sheep are susceptible to experimental infection with *N. caninum*, abortions in naturally infected sheep are not common (Spilovská et al., 2009).

In conclusion, unlike *N. canimum*, contact with *T. gondii* is common in sheep in the analyzed areas. Nevertheless, there is a need to determine additional risk factors for *Toxoplasma* infection in sheep besides their region of origin (da Silva et al., 2012) and to analyze in depth the reason for the differences in seroprevalnce observed among regions.

Declaration of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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