



Seroprevalence and risk factors associated with *Toxoplasma gondii* infection in pig farms from Catalonia, north-eastern Spain

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ABSTRACT

Seroprevalence and associated risk factors for *Toxoplasma gondii* infection in pigs were analyzed in 1202 sera samples, including sows and pigs of three, seven, 11, 15 and 20 weeks of age, from 23 farms in Catalonia, north-eastern Spain. Antibodies were tested by the modified agglutination test (MAT) at titers $\geq 1:25$. Antibodies to *T. gondii* were found in 228 samples (19.0%; 95% CI: 16.8–21.2). The individual prevalence in animals higher than 7 weeks of age was 22.8% (174/762; 95% CI: 16.6–29.0) and the within-farm prevalence ranged from 7.1% to 36.4%. Statistically significant differences were found among age classes. The risk factors significantly associated with *T. gondii* seroprevalence were the presence of cats, percentage of mortality at weaning and the presence of outdoor facilities in the farms. The seroprevalence observed in the present study indicates widespread exposure to *T. gondii* among domestic pigs in Catalonia, which may have important implications for public health.

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Domestic pigs (*Sus scrofa*) are considered an important source of *Toxoplasma gondii* infection in humans in several countries (Dubey and Beattie, 1988; Tenter et al., 2000; Dubey, 2009). Pigs can be infected with *T. gondii* through ingestion of food and water contaminated with sporulated oocysts, by consuming cysts contained in tissues of infected animals such as rodents, birds and other pigs, or congenitally (Dubey and Beattie, 1988; Dubey et al., 1995a).

Numerous serologic surveys on the prevalence of *T. gondii* in pigs from different countries have been carried out (Tenter et al., 2000; Dubey, 2009). More information is available on *T. gondii* infection in pigs from the USA where seroprevalence has decreased drastically in the last decade (Dubey and Jones, 2008). However, in European countries the recent trend of rearing pigs outdoors (e.g. organic farming) is likely to result in an increase of seroprevalence, as has been shown in pigs in the Netherlands (van der Giessen et al., 2007). There is no recent data on prevalence of *T. gondii* infection in pigs in Spain. The only study, by Aparicio-Garrido et al. (1972) whom reported antibodies in animals for food in Madrid, Spain, is more than 30 years old.

The aims of the present study were to determine individual prevalence of *T. gondii* antibodies in swine farms from Catalonia

(north-eastern Spain), to obtain the seroprevalence of *T. gondii* in different age classes, and to characterize the main associated risk factors to the presence of antibodies in this region. Catalonia is the largest swine-producing region in Spain (around 7231 swine farms and an estimated total census of six millions pigs) and accounts for over 25% and 4% of the Spanish and European swine production, respectively (Anonymous, 2008).

A cross-sectional survey was carried out in 23 swine farms in Catalonia during 2007 and 2008. The farms included in the study were randomly selected from the census of swine farms of Catalonia. The size of the farms included in the study ranged between 90 and 9500 pigs (mean = 2609) with a mean number of sows, fattening pigs and nursery-growing pigs of 450, 1020 and 1140, respectively. Only farrow-to-finish ($n = 16$) or piglet production farms ($n = 7$), with a number of sows >80 were included in the study. The number of samples collected for the different age groups was calculated with a 95% confidence and an accepted error of 5%. For sows, a high prevalence (30%) was assumed, while in the remaining age classes, an expected prevalence of 10% was assumed based on Dubey (2009), thus 323 samples needed to be collected for sows and 139 samples for each age group in piglets. Ten animals each of three, seven, 11, 15 and 20 weeks of age, and 14 sows were randomly selected in each farm (Table 1).

General information on the farm, production and health parameters, facilities and biosecurity measures data were collected using a structured filled-in questionnaire obtained by direct interview

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Table 1
Prevalence of *T. gondii* antibodies in pigs among age classes.

Age of pigs	No. examined ^a	No. positive	% prevalence	(95% CI)
3 Weeks	230	24	10.4	(6.5–14.4)
7 Weeks	210	30	14.3	(9.6–19.0)
11 Weeks	150	46	30.7	(23.3–38.1)
15 Weeks	150	53	35.4	(27.7–43.0)
20 Weeks	140	25	17.9	(11.5–24.2)
Sows	322	50	15.5	(11.6–19.5)
Total	1202	228	19.0	(16.8–21.2)

^a Twenty samples could not be collected from pigs at 7 and 20 weeks, 10 samples could not be collected from pigs of 11 and 15 weeks.

with each swine farmer. A total of 1202 blood samples were collected from the vena cava of young pigs and the tail vein of sows (Vacutainer®, Becton–Dickinson). The presence of antibodies to *T. gondii* in swine sera was tested by the modified agglutination test (MAT) as described previously (Dubey and Desmonts, 1987). Sera with a titer $\geq 1:25$ were considered positive.

Due to possible interference with colostrum-derived antibodies, only animals higher than 7 weeks of age were included to determine the estimate individual, within-farm and farm seroprevalence to *T. gondii*. To determinate the risk factors potentially associated to *T. gondii* infection, a multivariate analysis was conducted by means of lineal regression model using the SPSS 14.0 statistical program. On an individual level, the effects of independent variables (age and parity number) on seropositivity were analyzed by generalized linear mixed models (GLMM) using the statistical software package SAS 9.1. The number of *T. gondii* positive animals was assumed to follow a binomial distribution and the farm was considered as a random effect.

Antibodies against *T. gondii* were found in 228 (19.0%; 95% CI: 16.8–21.2) of 1202 pigs (Table 1) in titers of 1:25 in 158, 1:50 in 41, 1:100 in 18, 1:200 in 9, 1:800 in 1, and $\geq 1:1600$ in 1 pig. The overall estimate seroprevalence in animals higher than 7 weeks of age was 22.8% (174/762; 95% CI: 16.6–29.0). The within-farm prevalence ranged from 7.1% to 36.4% (mean: 25.2%; 95% CI: 20.2–30.2). The prevalence of infected farms was 87.0% (95% CI: 73.3–100). Statistically significant differences were found among the different age classes (Table 1). Eleven and 15 week-old pigs had significantly higher seroprevalence than the other age groups. The mean parity range of the analyzed sows was 2.9 ranging from zero to eight. No statistically significant differences in seroprevalence were observed among parity number in sows.

The factors significantly associated with *T. gondii* seropositivity in pig farms were: the presence of cats, percentage of mortality in weaning and presence of outdoors facilities for sows (Table 2). Based on the odds ratio, the likelihood of presence of *T. gondii* antibodies increased 11.3 times if cats were present in the farms and 9.7 times if the pigs had outdoor facilities. The likelihood of

Table 2
Results of the lineal regression analysis of the risk factors associated to *T. gondii* seropositivity in 23 pig farms from Catalonia, Spain.

Risk factor	No. examined (%)	Odds ratio	95% CI	B	p-Value
<i>Presence of cats</i>					
Yes	638 (24.8)	11.3	3.2–19.3	0.570	0.01
No	564 (12.4)	Reference			
<i>Outdoor facilities for sows</i>					
Yes	402 (22.9)	9.7	1.7–17.73	0.491	0.02
No	766 (17.6)	Reference			
<i>Mortality in weaning</i>					
>2%	482 (23.0)	2.3	0.9–3.7	0.543	0.01
<2%	504 (14.9)	Reference			

presence of *T. gondii* antibodies also increased as mortality in weaning increased.

The present results indicated that *T. gondii* is widely spread among pig farms in Catalonia. Wide variations in within-farm seroprevalence were observed, as have been reported in previous studies from other countries (Tenter et al., 2000; Dubey and Jones, 2008). Although the number of sampled farms in the present study was limited, the results obtained provide a first approximation to the farm prevalence in an area where there was no previous information. However, further studies including a higher number of farms are needed in order to obtain a more accurate estimation of farm prevalence in Catalonia.

Statistically significant differences were observed between seroprevalence levels and age. In pigs, IgG antibodies are not passively transferred in uterus, and seropositivity in colostrum deprived animals would indicate transplacental transmission and fetal infection (Dubey and Urban, 1990). However, in the present study, animals had ingested colostrum and therefore, antibodies in piglets of 3 and 7 weeks of age could have been due to the presence of maternal derived antibodies (Dubey and Urban, 1990). In agreement with this finding, the seroprevalences observed in piglets at 3 and 7 weeks of age were similar to the observed seroprevalence in sows. On the other hand, the high seroprevalence of *T. gondii* antibodies in 11 and 15 week old pigs could be an indication of horizontal infection in these age groups. In contrast, most studies have reported higher seroprevalence in breeding sows than fattening pigs (Dubey, 2009).

The fact that approximately 70% of seropositive pigs had a low (1:25) MAT titer could be indication of low levels of *T. gondii* exposure to the parasite in the analyzed farms. MAT has been validated in naturally infected pigs (Dubey et al., 1995b) and the parasite has been isolated from naturally infected pigs and wild pigs with antibody titres as low as 1:25 by MAT (Dubey et al., 1995a; Richomme et al., 2009, respectively). On the other hand, recent studies have shown that other serological tests (ELISA, immunoblotting) could also be good methods for the serological detection of *T. gondii* infection in pigs (Garcia et al., 2008).

The presence of cats on the pig farms was the main risk factor for *T. gondii* seroprevalence. Epidemiologic observations suggest that cats are essential for the maintenance of *T. gondii* infection in pig farms through oocyst elimination and contamination of feed and/or water (Dubey and Beattie, 1988; Weigel et al., 1995; Lehmann et al., 2003; Meerburg et al., 2006). In the present study, we did not examine cats or other wildlife on the farms, but in a previous study, a high rate (45%) of *T. gondii* infection was found in feral cats from Barcelona (Gauss et al., 2003), the main urban area of Catalonia. Therefore, an even higher prevalence could be expected in cats from rural areas of Catalonia that would affect seroprevalence levels in pig farms, as indicated in the present study.

The observed *T. gondii* seroprevalence was also significantly higher in farms with outdoors facilities for pigs, which has also been reported in previous studies (Assadi-Rad et al., 1995; Davies et al., 1998). The access to outdoor facilities may favor contact with cats and rodents, increasing the probability of ingestion of oocysts and tissue cysts, respectively (Venturini et al., 2004).

A significant positive association was observed between the percentage of mortality in weaning pigs and the prevalence of *T. gondii* antibodies. These findings are difficult to explain because toxoplasmosis rarely causes clinical toxoplasmosis in pigs (Dubey and Jones, 2008). Damriyasa et al. (2004) observed that the within-farm seroprevalence was significantly higher in sows with reproductive disorders than in those without such problems.

In summary, the results in the present study indicate widespread exposure to *T. gondii* among domestic pigs in north-eastern Spain and suggest an important role of cats in the transmission of

this parasite in swine farms from Catalonia. In order to decrease the risk of *T. gondii* infecting their pigs, farmers should control the presence of cats on their farms.

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