Seroepidemiological study of toxoplasmosis in sheep in rural areas of the Grosseto district, Tuscany, Italy

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Abstract

Six hundred and thirty individual serum samples from dairy sheep were tested for the presence of antibodies to Toxoplasma gondii using an indirect immunofluorescence antibody test (IFAT). The sampled animals came from 33 dairy herds representative of the southern area of the Tuscany region. Questionnaires exploring the management system were filled in by the veterinarian in charge of the herds. Using a cut-off of 1:64, 214 (OR=1.514, CI=1.050-2.182), and access of cats to water given to animals (OR=2.046, CI=1.284-3.261), still water source and access of cats in the farm, farm management (Masala et al., 2003; Klun et al., 2006; Samra et al., 2007; Pinheiro et al., 2009; Lopes et al., 2010).

In an attempt to characterize the risk of the diffusion of toxoplasmosis a study has been undertaken to estimate seroprevalence and risk factors associated to T. gondii infection in naturally exposed dairy sheep in Tuscany, Italy. This is the first time that a similar research is done in Tuscany, Italy, therefore no data are available in literature.

Materials and methods

Serum samples were collected from 630 adult sheep (>18 months old) of the sarda breed in 33 herds in the Grosseto district (Tuscany, Italy) during May-June 2011. The selected area extends approx from the latitude 42.851078° to 42.51902° and the longitude 11.129635° to 11.531657°. The area is mainly hilly and sampled farms extend from 13 to 574 m asl (mean=128.09, sd=114.20). Sample size was calculated using a 25% expected animal-level prevalence, 7.5% absolute error and 95% confidence level. This is the first time that a similar research is done in Tuscany, Italy, therefore no data are available in literature.

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Blood samples (venous blood) were always obtained from sheep in farms. Samples were transferred to the laboratory on ice and after centrifugation (3000 rpm for 10 min), the sera...
were stored at -20°C until analysis (Urquart et al., 2005). Serum samples were analyzed through a commercial indirect immunofluorescence antibody assay (IFAT) to investigate the presence of IgG antibodies against T. gondii. The serological test was performed according to the method of Camargo (1974) using slides spotted with whole RH strain tachyzoites (Mega Cor Diagnostic, Horbranz, Austria) as antigens and fluorescein isothiocyanate-labeled rabbit anti-sheep IgG (whole molecule, Sigma-Aldrich, St. Louis, MO, USA). In the kit insert, the manufacturer states that: the test shows a sensitivity in excess of 99% and specificity above 99%. True prevalence (TP) was calculated using standard methods \(\text{TP} = \frac{(AP+sp-1)/(se+sp-1)}{\text{where AP is the apparent prevalence, se ad sp are, respectively, the sensitivity and the specificity of the test}} \) (Bottarelli, 2012).

To identify risk factors associated with the infection of T. gondii, first a univariate analysis of the interest variables with the Pearson’s \(\chi^2\)-test or Fisher’s exact test, when necessary, was conducted. The association between each potential risk factor and seroprevalence was assessed using a Poisson regression model of the number of seropositive animals with the number of animals tested as an exposure variable. Variables associated with the outcome (P<0.20) were then entered into a multivariable Poisson model, with number tested as exposure. A multivariate logistic regression was then performed with StatView 5 for Mac OS (SAS Inst. Inc., Cary, NC, USA).

Results and discussion

At individual animal level, the seroprevalence was 33.97% (214 sample positive out of 630), 95% confidence interval [CI] 28.11 to 35.86%. Among the 214 reactive samples, 42 (19.63%) had an antibody titer of 1:64, 36 (16.82%) of 1:128, 39 (18.22%) of 1:256 and 97 (45.33%) of 1:512 or above. Using a sensitivity of 97.30% and a specificity of 96.00%, as claimed by the manufacturer, the overall seroprevalence of T. gondii was calculated as 32.12% (CI=28.47-35.77). When more cautious values, sensitivity of 80% and specificity of 95%, were assumed (Andreotti et al., 2007; Shaapan et al., 2008; Macri et al., 2009), TP was calculated to be 38.62% (CI=34.82-42.43). Using a 1:64 cut-off point, and if an individual herd is classified as positive on the basis of having one or more seropositive animals, then 96.97% of the herds in the area would be classified as positive. Using a more rigorous definition of herd positivity, at least four seropositive animals in a herd, a cautious interpretation when specificity is less than 100% (Adaska and Anderson, 2003), then 63.64% of the herds would be classified as positive (21 of 33 herds).

The distribution of seropositive sheep according to answers to the audit questionnaire and the corresponding multivariate logistic regression are shown in Table 1. Herd size (P<0.005), water source (P<0.05) and access of cats to water given to animals (P<0.05) were statistically associated with T. gondii seropositivity at animal level. Roaming cats were found in all farms and since one row is filled with zeros, \(\chi^2\)-analysis was impossible. Animal raised in small herds had an higher probability (O.R. 2.046, CI=0.284-3.261) of being infected by T. gondii than animals from large herds. Animal raised in farms with still water source had 1.514 (CI=1.050-2.182) times more chances of being infected by T. gondii than animals raised in farms with running water source, while animals in herds where cats have access to water for sheep had 1.585 (CI=1.057-2.379) more chances. Separate water troughs for young and adult animals, separate feeding troughs for young and adult animals, purchase of spare breeding animals in the last 5 years, presence of established cats in property and presence of cats in places where feed is stored had no influence (P>0.05) on the T. gondii serological status of sheep.

Lamb, mutton and sheep milk are an important source of human T. gondii infections, but actual data on the prevalence of T. gondii in sheep are scarce (Rinaldi and Scala, 2008; Cenci-Goga et al., 2011). The present study estimated the seroprevalence of T. gondii in sheep in Tuscany, Italy, and analyzed the risk factors associated to the infection. The test of choice was a commercial indirect fluorescent antibody test (IFAT). Although the variability of the sensitivity and specificity according to the Toxoplasma strain employed, and the subjectivity in interpreting the fluorescence reaction make it difficult to compare results from different laboratories, the IFAT is simple to carry out even for large number of samples and several dilutions (Cenci-Goga et al., 2011). Data in the literature show some variability even in the same regions and in the same type of farm (Piergili Fioretti, 2004; Shaapan et al., 2008; Macri et al., 2009). A recent study (Macri et al., 2009) has revealed that there is a low strength of agreement between IFAT and modified agglutination test (MAT) in cat and dog sera.

There has been an increasing interest in recent years on the prevalence of toxoplasmosis in small ruminants because of their role on the dissemination of the protozoan to man through direct contact or by consuming products of animal origin (Ueno et al., 2009; Lopes et al., 2010; Opsteegh et al., 2010; Alvarado-Esquivel et al., 2011; Rossi et al., 2011). Although the seroprevalence of T. gondii infection in the sheep population has been estimated for years to be at a median value of 30% (Blewett, 1983), very few studies have been conducted in Italy and little is known about the prevalence of T. gondii in areas where sheep farming is an important element of the local economy. The prevalence of more than 30% sheep seropositive for T. gondii infection demonstrated with this study is considered consistent when compared to the results obtained by other authors (Masala et al., 2003; Fusco et al., 2007; Zedda et al., 2010). Nonetheless, this result is different from those obtained by others (Natale et al., 2007; Vesco et al., 2010).

Table 1. Risk factors associated or not to T. gondii seroprevalence in sheep in the Grosseto district, Tuscany, Italy, results of a multivariate logistic regression.

| Herd size       | OR (95% CI) | P      |
|-----------------|-------------|--------|
| Large (>400)    | 1*          |        |
| Medium (300≤≤400) | 1.653 (0.999 - 2.737) | 0.0506 |
| Small (<300)    | 2.046 (1.284 - 3.261) | 0.0026** |
| Water source    |             |        |
| Running         | 1*          |        |
| Still           | 1.514 (1.050 - 2.182) | 0.0263** |
| Access of cats to water given to animals |     |        |
| No              | 1*          |        |
| Yes             | 1.585 (1.057 - 2.379) | 0.0260** |

OR, odd ratio. *Reference level; **P<0.05.
Conclusions

Given the results of this study, it can be assumed that herd size, water source, and access of cats to water are the risk factors for Toxoplasma infection in the sheep herds tested in the Grosseto district in Tuscany, Italy. Control and prophylactic measures must be adopted to improve the rearing system and the implementation of health promoting programs in a joint effort between sheep farmers, farmers’ associations and veterinarians to inform about the means of transmission of the disease and for a better understanding of the disease.

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Opsteegh, M., Teunis, P., Mensink, M., Züchner, L., Titilincu, A., Langelaar, M., 2007; Chikweto et al., 2011; Hutchinson et al., 2011), who reported higher prevalence. The variability of these results may be due to the differences in the age and management of the sampled animals, the environment and in the serological technique used (Piergilli Fioretti, 2004; Cenci-Goga et al., 2011). A positive association was observed between seroprevalence of T. gondii and the access of cats to water given to animals, indicating that the intimate contact with feline species is important in the epidemiology of toxoplasmosis. This could explain the high prevalence of T. gondii-specific antibodies observed in sheep, which was probably not due to direct contact with cats, given that no association was observed between seroprevalence of T. gondii and the presence of cats in the property, but rather to the elimination of oocysts by the cats that contaminated the environment. On the other hand, no association was observed between seroprevalence of T. gondii and access of cats in places where feed is stored. This finding is consistent to data reported previously (Skjerve et al., 1998) where the presence of a cat as such was not found to be a risk factor. Cats are, indeed, likely to be found in almost all areas where sheep are kept, and the probability that a roaming young cat may shed oocysts on a farm will always be present. As oocyst survival in soil for up to two years has been reported, any faecal material from infected cats will vival in soil for up to two years has been report-
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