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Research paper

Toxoplasma gondii seroprevalence in dairy and beef cattle: Large-scale epidemiological study in Estonia

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ABSTRACT

Toxoplasma gondii is a zoonotic protozoan parasite that thrives in Estonia. In this nationwide cross-sectional study, we tested sera from 3991 cattle, collected from 228 farms in 2012–2013, for anti-*T. gondii* immunoglobulin G antibodies using a commercial direct agglutination test. Titer of 100 was set as cut-off: samples that tested positive at the dilution 1:100 were defined as positive. The apparent animal-level seroprevalence was 18.62%. At least one seropositive animal was found on 68.86% of the farms, and seropositive cattle were detected in all counties. The seroprevalence appeared to increase with age until five years (60–71 months) of age, but had no obvious pattern in the older animals. Animals of the local Estonian Red breed had higher odds to test seropositive than did animals of the Estonian Holstein breed. Whether the farm focused on dairy or beef cattle was not associated with an animal testing *T. gondii* seropositive nor with finding at least one *T. gondii* seropositive animal on the farm. The odds of finding at least one *T. gondii* seropositive animal on the farm were higher if the herd size was above median (105 in dairy and mixed dairy farms; 35 in beef and mixed beef farms). The results indicate that *T. gondii* is endemic within the agricultural setting in Estonia and present on the majority of cattle farms.

1. Introduction

Toxoplasma gondii is a zoonotic protozoan parasite, which has been ranked high among foodborne pathogens (FAO/WHO, 2014). The role of cattle in the epidemiology of *T. gondii* is not well described, while undercooked meat and raw milk derived from infected animals are well-known sources of the infection to other hosts (Tenter et al., 2000; FAO/WHO, 2014).

In Estonia, which is located in northeastern Europe, no specific measures have been taken to prevent *T. gondii* infections in any of its host species. The seroprevalence is high (55.8%) in the human population (Lassen et al., 2016) as well as in the relevant definitive hosts, domestic cats (60.8%) (Must et al., 2015). Because seropositive cats are considered to have shed oocysts and more than half of the cats had outdoor access, it is likely that the environment is contaminated with *T. gondii* oocysts. This is supported by almost a quarter (24.0%) of investigated wild boars, which likely acquire the infection from the soil, testing seropositive (Jokelainen et al., 2015). Cattle and other herbivorous domestic animals likely encounter *T. gondii* from the environmental oocyst reservoir, either on farm or pastures, or via contaminated water or feed.

Our hypothesis was that serological evidence of exposure to *T. gondii* is relatively common in cattle in Estonia. Our cross-sectional study aimed to estimate *T. gondii* seroprevalence in cattle in Estonia and to evaluate possible risk factors for *T. gondii* seropositivity at animal-level and at farm-level.

2. Materials and methods

2.1. Ethics statement

No animals were sampled for the purpose of the present study; we used aliquots from samples taken for unrelated national surveillance and eradication program. All information regarding farms was treated confidentially. Serum samples and farms were coded, and serology was performed blinded.

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2.2. Study setting and design

According to the animal register of the Estonian Agricultural Registers and Information Board, there were 247,510 cattle in Estonia in 2012 (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014). The present study was a nationwide cross-sectional serological study of naturally-acquired bovine *T. gondii* infections. Our target population was adult cattle on dairy and beef farms in Estonia.

2.3. Study population and sampling frame

The study population comprised of cattle from herds with at least five cows sampled under the official surveillance and eradication program for enzootic bovine leukosis. All cows, heifers, and bulls aged ≥ 24 months were to be tested annually for enzootic bovine leukosis antibodies (Riigiteataja, 2010). Milk samples were collected from dairy cows lactating at the time of sampling and blood samples were collected from other cattle. The blood samples were collected by the state veterinary service into sterile vacutainers without anticoagulant. From each farm, 5–30 sera were randomly selected for further studies and stored at -20 °C. The sampling frame available for this study included the sera aliquots from 9170 cattle, originating from 489 farms and collected between February 28th 2012 and March 31st 2013 (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014).

2.4. Sample size calculations

Sample size calculations were performed with OpenEpi (Dean et al., 2015) and the EpiTools calculator 'sample size for 2-stage prevalence survey' (Sergeant, 2016).

The available sample was evaluated as suitable for an overall estimate of animal-level seroprevalence: with expected *T. gondii* seroprevalence of 10%, intra-cluster correlation coefficient of 0.2, sampling 30 animals per farm, and a confidence level of 95% to 99%, the minimum sample size needed would be 945–1,625 samples (Dohoo et al., 2009). The sampling frame allowed two-stage sampling, and the number of samples to allow estimating the seroprevalence separately for dairy farms and beef cattle farms was calculated using 0.05 between-cluster variance, 5% accepted error limit, and 95% confidence level. On farms with at least five cattle, the mean cluster size among 1161 dairy herds was 78 animals and among 471 beef herds 19 animals. The minimum number of farms to sample was 77 per stratum with 21 animals from each farm: 1617 in total per stratum. To account for the possibility that some samples could be missing and that 5–30 animals would be included per farm to include also smaller herds, 125 farms were included per stratum.

2.5. Selection of samples

Two-stage sampling was used, with farms as the primary and animals as the secondary sampling units. From the farms of the original serum bank, 125 farms originally classified as dairy farms and 125 farms originally classified as beef farms were randomly selected (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014), using a random number generator (Excel, Microsoft Office 2013, Microsoft Corporation, Washington, USA). From each selected farm, the sera from 5 to 30 animals were included. Few animals were excluded due to no or insufficient amount of serum (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014).

2.6. Serology

The sera were screened for specific anti-*T. gondii* immunoglobulin G (IgG) antibodies using a commercial direct agglutination test (DAT; Toxo-screen DA, bioMérieux, Marcy-l'Étoile, France), following the manufacturer's instructions, with the exception of sample dilution. We set titer of 100 as cut-off: the sera were diluted 1:100, and those testing positive were defined positive (Dubey et al., 1985; Lopes et al., 2013). The results were read after 18 h and recorded using a four-point scale (Jokelainen, 2013), followed by further interpretation as either seropositive or seronegative. Only samples with clear positive results were considered positive. The positive and negative controls provided by the manufacturer were included on each plate at dilutions 1:40 and 1:4000. The antigen control, including all the reagents except serum, was performed on each plate.

2.7. Source and exclusion of data

Information for each animal and farm were obtained from the Estonian Agricultural Registers and Information Board. From farms that had been sampled more than once, the results of the first sampling timepoint were retained in the analyses (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014).

2.8. Re-classification of the farm types and generation of two datasets

Because a substantial proportion of the farms had cattle of both dairy and beef breeds, the farms were re-classified into four groups (see Fig. S1 in the online version at DOI http://dx.doi.org/10.1016/j.vetpar.2017.02.014). Farms with ≤ 5% cattle of beef breeds were defined as dairy farms, while farms with ≤ 5% cattle of dairy breeds were defined as beef farms. Farms with mainly cattle of dairy breeds but > 5% cattle of beef breeds were defined as mixed dairy farms, while the farms with mainly cattle of beef breeds but > 5% cattle of dairy breeds were defined as mixed beef farms. Two datasets were compiled for the risk factor analyses: the first comprised data of animals from dairy and mixed dairy farms and the second of animals from beef and mixed beef farms.

2.9. Variables

The animal-level variables were 'age' (in months and categorized according to quartiles) and 'breed', and the farm-level variables were 'farm type' (dairy, beef, mixed dairy, mixed beef), 'herd size' (categorized at median and according to quartiles), and the 'county' in which the farm was located. Moreover, variable 'region' was created: North West (Raplamaa, Harjumaa, Läänemaa, Hiiumaa), North East (Lääne-Virumaa, Ida-Virumaa, Järvamaa, Jõgevamaa), South West (Viljandimaa, Pärnumaa, Saaremaa), and South East (Tartumaa, Põlvamaa, Valgamaa, Võrumaa). For descriptive statistics, age was also categorized by six months and years, and the herd size at 50, 100, 200, and 400 animals.

2.10. Outcomes

The animal-level outcome was dichotomous: each individual animal was defined as either *T. gondii* seropositive or seronegative. The farm-level outcome was dichotomous: each farm had either at least one seropositive animal or no seropositives.