Toxoplasma gondii in naturally infected goats: ~~m~~Monitoring of specific IgG levels in serum and milk during lactation and parasitic DNA detection in milk

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Abstract

The zoonotic protozoa *Toxoplasma gondii* is one of the major abortive pathogens in small ruminants. Nevertheless, data on *T. gondii* infection in goats during lactation and on the presence of *T. gondii* in goat milk are lacking. A longitudinal study was planned in a *T. gondii* naturally infected dairy goat farm with the aim of (i) evaluating the variation of anti-*T. gondii* antibodies in blood and milk during the lactation; (ii) identifying the optimal phase during lactation for *T. gondii* monitoring; (iii) detecting the presence of *T. gondii* DNA in the milk. From March to July 2017, 30 goats in a farm were fortnightly visited seven times and sampled for blood and, when in lactation, for milk. Individual data regarding age, reproductive disorders, and the day of lactation were recorded. For the detection of anti-*T. gondii* antibodies in blood and milk a commercial ELISA kit was used. Milk samples ($n = 63$) of selected nine seropositive animals were also molecularly analysed to amplify a sequence within the ITS1 region of *T. gondii*. The seroprevalence of *T. gondii* infection was 63.3% (19/30); a high agreement was obtained between serum and milk results (Spearman's coefficient = 0.793 and Kendall's tau = 0.624), particularly between the 15^{th} and the 60^{th} day of lactation. In the statistical analysis, performed with generalized linear mixed models (GLMMs), the variable “phase of lactation” was strongly associated to ELISA values obtained in both serum and milk (p-value = 0.0001, $F = 5.197$, and p-value = 0.016, $F = 2.755$, respectively). Finally, molecular analyses revealed the presence of parasitic DNA in 20.6% (13/63) of milk samples, with a discontinuous parasite excretion; statistical analyses did not reveal any association among the parasite excretion and the considered variables. Milk could be considered as a valid alternative to blood for monitoring *T. gondii* infection in goat herds. Moreover, the detection of *T. gondii* DNA in milk enhanced the possibility for raw goat's milk consumption to be considered as a risk to public health.

Keywords: *Toxoplasma gondii*; ELISA; Milk; Serology; PCR; Italy

1 Introduction

Toxoplasma gondii is an Apicomplexa protozoan showing a wide range of hosts and a worldwide distribution (Dubey, 2009). Toxoplasmosis in humans still represents a public health issue; it is considered as the most prevalent zoonotic parasite worldwide and the third food-borne pathogen in Europe (EFSA Panel on Biological Hazards, 2018). The consumption of raw or undercooked meat is regarded as one of the major risks of acquiring *T. gondii* infection for humans (EFSA, 2007). Among the possible infected meat, small ruminant products, particularly from goats, are a major source of *T. gondii*, mostly for those countries and ethnic groups for which the consumption of goats’ undercooked meat is a cultural and traditional habit (Kijlstra and Jongert, 2008). In addition, the consumption of raw milk from *T. gondii*-infected goats has been proposed as a possible contamination route for humans (Boughattas, 2017). Indeed, human toxoplasmosis outbreaks and cases of infection linked to the consumption of raw goat milk have been reported (Sacks et al., 1982; Chiari and Neves, 1984).

Besides its importance from a public health viewpoint, *T. gondii* also has a zootechnical relevance being one of the major abortive pathogen in small ruminants (Ortega-Mora et al., 2007). Several epidemiological studies reported high seroprevalence among goats, up to 60% in European studies (Iovu et al., 2012; Tzanidakis et al., 2012; García-Bocanegra et al., 2013; Lopes et al., 2013; Gazzonis et al., 2015).

At the herd level, the identification of *T. gondii* infection through antibody detection allows the planning of health actions to reduce the percentage of seropositive animals (Bartels et al., 2007). With the aim of reducing costs and animals’ stress related to blood sampling, several serological tests have been developed and standardized for antibody detection in milk samples for many pathogens (Sekiya et al., 2013). Particularly, a commercial ELISA kit has been recently validated for the detection of specific IgG anti-*T. gondii* in goat milk (Gazzonis et al., 2018a). Nevertheless, little information is available on antibody kinetics in goats during lactation (Ferrer et al., 1997; Levieux et al., 2002), and thus on the optimal phase of lactation to carry out milk sampling for antibody detection.

Likewise, little is known about the excretion of *T. gondii* in milk. *T. gondii* DNA was detected by molecular tools in goat milk samples with prevalence ranging from 1 to 32.5% (Spišák et al., 2010; Mancianti et al., 2013; Bezerra et al., 2015; da Silva et al., 2015). Several studies, meta-analysed by Boughattas (2017), have determined the consumption of raw milk, especially of goat origin, as a risk factor enhancing the risk of *T. gondii* infection. However, the real effectiveness of this transmission route for humans is yet to be defined.

Therefore, a longitudinal study on *T. gondii* infection in a naturally infected dairy goat herd was planned with the aim of (i) investigat~~e~~ing the immune response against *T. gondii* in goats during the lactation, (ii) assessing the optimal physiological period to detect antibodies in milk and therefore to evaluate the optimal phase of lactation to monitor *T. gondii* in a goats' herd through analyses on milk; (iii) investigat~~e~~ing the presence of *T. gondii* DNA in milk and its possible variation during lactation.

2 Material and methods

2.1 Ethical statement

The collection of biological samples from live animals was performed by veterinarians, following good clinical practices in the respect of animal welfare according to current Italian legislation. The study was conducted with the approval of the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_34_2017).

2.2 Study design

A longitudinal study in a case-study goat's herd was carried out. The farm was visited fortnightly from March to July 2017 (seven visits: T1-T7), corresponding to the *peripartum* period through the lactation for the majority of goats. Heats were indeed not synchronous in the group, and thus deliveries occurred at different times. The first two births occurred in February and the last one in June. For each animal, individual data concerning age and previous reports of reproductive disorders were collected; also, the day of lactation (DL) was calculated for each goat taking the day of birth as day 0.

2.3 Herd description and animal housing

The study was carried out in a family-run dairy goat farm in Varese province (Lombardy region, Northern Italy, 46°03′N; 8°48′E, 670 m a.s.l.) consisting of 30 Alpine female goats and a buck. All female goats aged between 13 and 87 months (mean ± s.d.: 46.7 ± 22.9) were included in the study. The herd was selected among previously surveyed farms: it resulted indeed positive for *T. gondii* infection (herd prevalence: 61.2%) and negative for *Neospora caninum* infection in two previously published epidemiological serosurveys carried out in ovine and caprine farms in Lombardy (Gazzonis et al., 2015, 2016).

The farmer produces cheese from milk in the farm; milk and products are sold at local marketplaces. This kind of traditional farming is typical of caprine breeding in Northern Italy and contributes to the safeguard of the economy of mountain territories otherwise often abandoned. Apart from two crossbreed goats, all animals in the farm belonged to Alpine breed. In the breeding season in which the study was carried out, the veterinarian practitioner observed some reproductive disorders in eight animals: abortion at different stages of pregnancy, repeated heats or failed insemination.

2.4 Sample collection

Blood samples (n = 210) were collected from all 30 animals at each of the seven sampling time. Since heats and deliveries were not synchronous, only 151 milk samples were collected: for some goats not all seven milk samples were available because they were not lactating at the sampling time.

Approximately, 10 ml of blood sample were collected in Vacutainer® tubes without anticoagulant agents from the jugular vein of each animal. Contemporary, after disinfection of teats and using latex gloves, 10 ml of milk samples were collected in sterile tubes from milking goats by manual milking. Samples were immediately transported refrigerated to the laboratory. Blood samples were centrifuged at 2120 ~~g~~ *g* for 15 min, at room temperature and obtained sera were stored at −20 °C until analysed. Milk samples were divided into two aliquots: the first one, for molecular analyses, was stored at −20 °C until analysed. The second one, for antibody detection, was processed to eliminate the fatty components and the somatic cells (Petruzzelli et al., 2013), then stored at −20 °C until analysed.

2.5 *Toxoplasma gondii* antibody detection in serum and milk samples

A commercial ELISA kit (ID Screen® Toxoplasmosis Indirect Multi-Species, IDVET, Montpellier) was used for analysis on both sera and milk. Serum and milk samples were analysed according to the manufacturer's instructions and according to the protocol described by Gazzonis et al. (2018a), respectively. Positive and negative control sera provided with the kit were used as controls both for serum and milk samples. Absorbance was measured as optical density (OD) values at 450 nm using a microplate reader (Multiskan Ascent 96/384 plate reader; MTX Lab Systems, Inc, Vienna, Virginia). For each observation, sample to positive ratio (S/P%) was calculated applying the formula supplied by the manufacturer:

S/P% = 100 × (OD sample - OD negative control)~~/~~/(OD positive control - OD negative control).

Serum samples with SP% ≥50% were considered positive, whereas the cut-off value for milk samples was set at 21.8% (Gazzonis et al., 2018a).

2.6 DNA extraction and molecular analyses on milk samples

To investigate the variation of *T. gondii* DNA excreted in milk, 63 milk samples from nine seropositive goats that were lactating during the whole study period were collected; for these selected animals, both serum and milk samples were available for each sampling time.

To avoid interference with casein, milk samples were pre-treated as described by Mancianti et al. (2013) with 200 µl TE (1 mM EDTA, 10 mM Tris~~-~~–HCl, pH = 7.6) and 300 µl 0.5 M EDTA (pH = 8), then processed for DNA extraction using a commercial kit (Nucleospin tissue, Macherey-Nagel, Germany). Extracted DNA was stored at −20 °C until analysed.

Samples were assayed for *T. gondii* DNA by a single tube nested-PCR amplification targeting a 227 bp sequence within the ITS1 region (Hurtado et al., 2001). Positive (Zanzani et al., 2016) and negative controls (no template DNA) were included in each PCR run. PCR products were run in 1.5% agarose gel containing 0.05% ethidium bromide in TBE buffer electrophoresis and visualized under UV light on a transilluminator. Bands of the expected size were excised from the gel, purified with a commercial kit NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel, Germany), and sent for bidirectional sequencing to an external laboratory (Eurofins MWG, Munich, Germany). Electropherograms of obtained sequences were checked, and consensus sequences obtained. Sequences were then compared to those available in publicly accessible databases using BLASTn software (https://www.ncbi.nlm.nih.gov/blast/).

2.7 Statistical analysis

Descriptive statistics were calculated as (i) estimated prevalence on results obtained in ELISA performed on both serum and milk samples and on results obtained by molecular analysis, and (ii) arithmetical means and standard deviation of S/P% ELISA results for data obtained in serum and milk samples.

The normality of ELISA S/P% values obtained in both serum and milk was assessed by plotting histograms and running normal tests (Shapiro–Wilk and Kolmogorov–Smirnov tests). Considering both results above and below the cut-off values, the distribution of ELISA S/P% values obtained both on serum and milk samples did not achieve normality (Shapiro–Wilk: *p*-values <0.0001 and <0.0001, and Kolmogorov–Smirnov: *p*-values = 0.001 and <0.0001, for serum and milk, respectively), thus non-parametric tests were applied when data from both seropositive and seronegative samples were analysed. Considering only seropositive samples, the normal distribution of ELISA S/P% values obtained both on serum and milk samples was achieved (Shapiro–Wilk: *p*-values = 0.045 and 0.134, and Kolmogorov–Smirnov: *p*-values = 0.2 and 0.2, for serum and milk, respectively).

To confirm the correspondence between sera and milk results, Spearman and Kendall rank correlation coefficients were computed. To verify if the agreement between results on serum and milk differed throughout the lactation, the same analysis was repeated dividing samples according to the phase of lactation: 0~~-15 DL, 16-30 DL, 31-45 DL, 46-60 DL, 61-75 DL, 76-~~–15 DL, 16–30 DL, 31–45 DL, 46–60 DL, 61–75 DL, 76–90 DL, and >90 DL.

Subsequently, it was verified if the phase of lactation could influence IgG levels in serum and milk. Only seropositive animals were considered (107 observations from 19 goats) and analysed through generalized linear mixed models (GLMMs). Two GLMMs were run using ELISA S/P% values obtained on serum and milk samples (continuous variable) as dependent variables, respectively.

The following response variables were entered in the models: “phase of lactation” (ordinal variable, seven categories as described above), “age” (continuous variable, expressed in months), and “problem in fertility/reproductive disorders” (binomial variable, presence/absence).

Finally, a third GLMM analysis was performed to verify if the presence of *T. gondii* DNA in milk could be influenced by any of the considered variables. The presence/absence of parasite DNA in milk was used as the response variable, and the following independent variables were entered in the model: phase of lactation, ELISA S/P% values in sera or milk, age, reproductive disorders.

For all GLMMs, a complete model containing all effects and their two-way interaction was firstly run. Subsequently, models were developed by backward elimination considering the goodness of fit with the Akaike information criterion corrected (AICC), until all remaining variables were significant (*p*-value<0.05). In the final, best fitting model, the estimated means of retained variables were then compared through pairwise comparisons. Goat ID and sampling time were entered in all models as nested random effects. Statistical analysis was performed using SPSS (version 19.0; SPSS, Chicago, Illinois).

3 Results

3.1 *Toxoplasma gondii* antibody detection in serum and milk samples

The results on ELISA analysis in sera samples revealed a *T. gondii* seroprevalence of 63.3% (19/30). Seronegative goats did not seroconvert during the whole survey period, except for two animals (Gt16 and Gt25), that scored seropositive only in two (Gt16-T2 and Gt16-T6) and one (Gt25-T6) sampling, respectively, with S/P% values slightly above the cut-off value. Similar results were obtained in the analysis of milk, with only five serum-milk pairs not in accordance. Two positive serum samples (with S/P% values slightly above the cut-off value: 50.2 and 53.2%, respectively) had the correspondent milk samples negative, while two negative serum samples had the correspondent milk samples scoring positive.

The correlation between S/P% values obtained in serum and milk samples was high (Spearman's coefficient = 0.793, *p*-value = 0.0001 and Kendall's tau = 0.624, *p*-value = 0.0001). The best agreement was obtained from the 15~~th DL until the 60th~~th DL until the 60th DL, while the worst at the beginning and the end of lactation (Table 1).

Table 1 Variation of the agreement between ELISA S/P% results obtained from lactating goat's serum and milk samples.

alt-text: Table 1

	Days from parturition						
Statistical test	0~~-1516-3031-4546-6061-7576-~~–15	16–30	31–45	46–60	61–75	76–90	>90
Kendall's Tau (*p*-value)	0.570 (0.0001)	0.713 (0.0001)	0.817 (0.0001)	0.630 (0.0001)	0.663 (0.0001)	0.748 (0.0001)	0.464 (0.003)
Spearman's coefficient (*p*-value)	0.675 (0.0001)	0.878 (0.0001)	0.948 (0.0001)	0.803 (0.0001)	0.796 (0.0001)	0.873 (0.0001)	0.580 (0.006)

Indeed, our results indicated a different trend in antibodies level in serum and milk: ELISA S/P% values were high both in serum and milk samples in the first fortnight, then in the following month (16~~-~~–45 DL) S/P% values increased even more in sera but decreased in milk. Seric IgG began to decrease 46 days after the beginning of lactation. Low ELISA S/P% values were registered both in serum and milk from the 46~~th~~th day of lactation, and subsequently immunoglobulins increased again both in sera and milk at the end of lactation (Fig. 1).

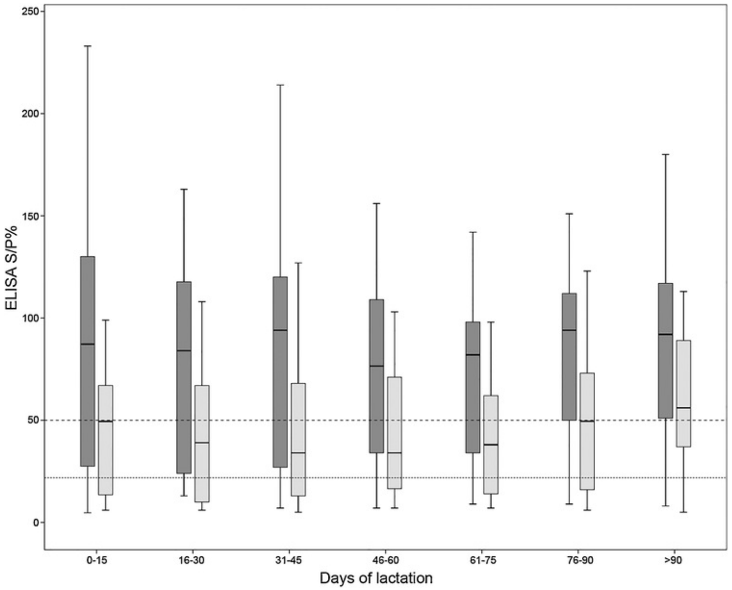

Fig. 1 ELISA S/P% values obtained in serum (dark grey) and milk (light grey) samples, during the lactation (computed in days of lactation).

Values above 50 (dashed line) and 21.8 (dotted line) are considered positive in serum and milk, respectively.

alt-text: Fig. 1

Statistical analysis confirmed the described trend: the variable "phase of lactation" was the only one retained in final GLMMs run on ELISA S/P% results obtained in serum and milk (p-value = 0.0001, F = 5.197, and p-value = 0.016, F = 2.755, respectively). The other considered variables were not statistically significant and were eliminated from the final models. In Table 2 descriptive statistics and results of statistical analysis are resumed; notably, pairwise comparison carried out on the estimated mean of ELISA S/P% results during the phases of lactation showed a clear difference in IgG values both in serum and milk between the first three fortnights (until 45th DL) and the rest of the lactation period.

Table 2 ELISA S/P% values in goat's serum and milk samples and results of generalized linear mixed models (GLMMs). Only seropositive animals were considered (107 observations from 19 goats included in both models).

alt-text: Table 2

	Serum			Milk		
Days *postpartum*	S/P%§ ± S.D.*	B ± S.E. #	p-value $	~~$~~S/P%§ ± S.D.*	B ± S.E. #	p-value $
~~$0-15112.1~~ 0–15	112.1[a] ± 30.2	21.0 ± 8.0	**0.01**	64.1 [abc] ± 20.1	~~-9.4-6.40.14616~~ −9.4 ± 6.4	0.146
16–30	110.0 [ab] ± 29.7	14.0 ± 7.0	0.051	60.3 [ab] ± 25.0	~~-~~−15.0 ± 5.5	**0.008**
31~~-~~–45	120.9 [a] ± 39.1	16.6 ± 6.4	**0.011**	61.6 [a] ± 28.7	~~-~~−16.8 ± 4.9	**0.001**
46~~-~~–60	97.0 [c] ± 33.4	1.9 ± 6.0	0.754	56.9 [a] ± 25.8	~~-~~−15.0 ± 4.7	**0.002**
61~~-7592.5 c 22.8~~ –75	92.5 [c] ± 22.8	−2.8 ± 5.2	0.580	56.2 [ab] ± 19.3	~~-~~−14.1 ± 4.4	**0.002**
76~~-~~–90	101.4 [c] ± 24.9	1.2 ± 5.6	0.828	62.2 [bc] ± 28.3	~~-~~−6.8 ± 4.4	0.164
>90	107.6 [bc] ± 35.6	0	–	~~-~~71.8 [bc] ± 24.4	0	–

§~~-~~ For each GLMM, ELISA S/P% values per each phase of lactation with different superscript letters (a, b, c) are statistically different from each other (p−value<0.05, pairwise comparison).

* **S.D.:** standard deviation; # **S.E.:** standard error; $~~$~~**:** statistically significant values are indicated in bold.

3.2 Molecular analysis

The results on the presence of *T. gondii* DNA in milk samples from nine selected seropositive goats were considered. *T. gondii* DNA was detected in 13 milk samples out of 63 examined (20.6%). Sequencing of PCR amplicons resulted in a group of identical sequences with the confirmation of the identity of *T. gondii* (homology of 100%). Only one goat did not show any positivity in PCR, while the other eight examined goats showed *T. gondii* DNA in milk at least in one sampling time. The highest number of PCR amplifications was achieved in the second fortnight of lactation (three positives out of eight examined samples in the phase 16–30 DL) and at the end of lactation (five positive samples out of 14 tested in the phase >90 DL). A lower DNA detection was achieved in the phases comprised between the 31st and the 90th DL; none out of five milk samples scored positive in the first fortnight of lactation (Table 3).

Table 3 Results of molecular detection of *T. gondii* DNA by nested-PCR in milk samples of selected nine seropositive goats, according to the phase of lactation (expressed in days of lactation).

alt-text: Table 3

	Days *post-partum*						
	0~~-1516-3031-4546-6061-7576~~ –15	16–30	31–45	46–60	61–75	76–90	>90
Positive/examined	0/5	3/8	2/9	1/9	1/9	1/9	5/14

Moreover, *T. gondii* DNA was detected in milk samples from animals showing a wide range of ELISA S/P% values both in serum and milk, ranging from 50.1 to 179.6% and from 24.9 to 103.5%, respectively. Results obtained in ELISA on serum and milk samples and data on the excretion of *T. gondii* DNA in milk samples are available at Mendeley Data (doi:10.17632/s98 × 6mmc2d.1). Statistical analyses did not show any association between the excretion of *T. gondii* in milk and the phase of lactation. Similarly, the presence of *T. gondii* DNA in milk seemed not to be influenced by any of the investigated variables and all variables were removed from the final GLMM.

4 Discussion

Toxoplasma gondii infection is a sanitary issue having a high economic impact on goat farming; particularly in the study area, Northern Italy where high seroprevalence rates were recorded in small ruminant farms, with 41.7 and 59.3% seropositive goats and sheep, respectively (Gazzonis et al., 2015). In addition, as a proof of the high environmental spread of the pathogen, in the same study area, *T. gondii* showed to be widely spread also in other domestic (Gazzonis et al., 2015~~; Gazzonis et al.,~~ , 2018b; Villa et al., 2018) and wild animals (Gazzonis et al., 2018c, d), with values varying according to investigated host species and on considered farming conditions.

In the present study, we investigated anti-*Toxoplasma gondii* IgG levels during lactation in serum and milk samples in a naturally infected dairy goat farm. The herd was selected having a chronic *T. gondii* infection within the herd, with a >60% intra-herd seroprevalence, and presenting history of fertility problems. The aim of the present study was to collect information about the dynamics of specific antibody levels both in sera and milk; moreover, the presence of *T. gondii* DNA in milk was investigated.

A commercial validated ELISA (Gazzonis et al., 2018a) was used for testing serum-milk pairs, with an optimal agreement between the results obtained in the two biological matrices. Collecting milk is easier and less expensive than collecting serum samples, as well as less stressing for animals. It can be used as a valid tool for a first approach to the screening of toxoplasmosis at the farm and individual level (Schares et al., 2004).

The concordance between sera and milk was calculated considering the different phases of lactation: the best agreement was obtained from the fifteenth day of lactation up to the 45^{th}. Indeed, obtained results showed that the phase of lactation is a risk factor influencing the antibody level both in serum and milk samples; nevertheless, few data are available regarding the physiological immunoglobulin levels in goats' milk during lactation. According to our data, IgG level was high in the first two weeks after birth in both serum and milk samples; subsequently, in milk, it decreased and showed a second peak from the 75^{th} DL. Conversely, in sera, high antibody levels were maintained for a more extended period until the 45^{th} day post parturition, then decreased and finally raised again at 75^{th} DL as in milk.

The first IgG peak in the first fortnight of lactation was already described; Ferrer et al. (1997) and Levieux et al. (2002) reported high IgG values in the first three days post-delivery. Afterwards, the concentration of immunoglobulins decreased, corresponding to the first 24~~-~~–36 hours after birth in which the intestine of new-borns goats can adsorb immunoglobulins from milk (Mesquita et al., 2013). The second peak we registered at the end of the study period may correspond to a phenomenon observed in other species (i.e., cattle). An increase of IgG levels was reported at the end of lactation, due to a decrease in milk yield and consequently to a higher milk protein and IgG concentration (Schares et al., 2004; Chanlun et al., 2006). The curve of antibody level in milk partially reflects that in serum, therefore, the IgG trend in milk during lactation may correspond to the variation of the systemic immunoglobulin production, although further studies are necessary to describe the recorded differences. From a diagnostic viewpoint, it could be inferred that analysing milk samples during the first fortnight from the parturition allows getting the most likely results to those obtained on the sera.

However, it must be considered that these data were obtained in a naturally infected herd and therefore in uncontrolled conditions. Some animals had reproductive disorders, such as abortions, repeated heats, or failed insemination. Although statistical analysis did not reveal any association between the presence of these alterations and the results obtained in ELISA neither on serum nor on milk, these conditions could be associated with a decrease in the immunocompetence of the goats, or with a different yield in terms of milk production with consequent alteration in the concentration of IgG in milk. Therefore, it would be desirable to reproduce the study design in experimentally infected animals, to follow the progress of the infection under controlled conditions.

In relation to the detection of parasite DNA during lactation, nine seropositive goats having serum-milk pairs for the all study period (i.e., already in lactation at T1) were considered, for an overall of 63 milk samples. All nine animals but one showed at least one positive *T. gondii* PCR milk sample. Parasite DNA excretion is thus discontinuous; particularly, the highest excretion of parasite DNA in milk was recorded at the beginning and the end of the study period, although this result was not supported by statistical analysis.

Camossi et al. (2011) evaluated the presence of *T. gondii* DNA in twenty ewes in the first two months of lactation: *T. gondii* DNA was detected in 10% of 70 milk samples from seropositive animals, i.e., the parasite DNA excretion was discontinuous as in our findings. Similarly, Dubey et al. (2014) reported an intermittent excretion of *T. gondii* DNA in eight experimentally infected goats. Furthermore, other studies described that only a part of the infected animals excrete the parasite DNA in milk: from a seroprevalence of 60.6% similar to those described in our study, Mancianti et al. (2013) reported 10 PCR positive milk samples out of 77 seropositive goats, while da Silva et al. (2015) detected *T. gondii* DNA in five goats' milk samples out of 186 analysed. Finally, Amairia et al. (2016)~~Aimaira et al. (2016)~~, out of 77 examined, found only six positive milk samples in PCR, two of which from seropositive goats.

The detection of *T. gondii* DNA in caprine milk, while supporting the hypothesis of parasite transmission through the consumption of raw milk or dairy products, does not necessarily demonstrate infectivity of milk to other animals. The vital stage of *T. gondii* that is presumably excreted by the infected host in milk is the tachyzoite, which is less resistant than oocysts or bradyzoites. Indeed, *T. gondii* tachyzoites are inactivated by pasteurization and low pH of gastric secretions (Dubey, 2009). Recently, an experimental study showed the ability of tachyzoites to resist in the gastric environment, with a further increase in survival in the case of addition of cow's milk (Koethe et al., 2017). Dubey et al. (2014) obtained similar findings for fresh cheese made from caprine raw milk contaminated with cell-cultured tachyzoites, with the survival of *T. gondii* during the cheese making process using cold enzyme treatment.

The consumption of raw goat's milk is therefore confirmed to represent a risk to public health, with the possibility of transmission, among other pathogens, also of *T. gondii*. Another possible risk is represented by the consumption of fresh cheeses made from raw milk of infected animals, although further studies in this sense are necessary.

An increase in selling and consumption of raw milk and unpasteurized dairy products has been recorded in recent years (EFSA Panel on Biological Hazards, 2015). Indeed, raw milk is often perceived by consumers as healthier and having greater health benefits than pasteurized milk, despite the possibility of transmission of pathogens (Lucey, 2015). Particularly, the consumption of goat's milk is increasing, especially among children suffering of allergy or intolerance to cow's milk. The casein present in goat's milk is similar to that present in human milk, therefore being more digestible and better tolerated by patients with lactose intolerance (Park et al., 2007). In Italy, the production of goat's milk is increasing, with a percentage increase reported of +40.5% from 2007 to 2017 (National Institute of Statistics ISTAT, http://dati.istat.it). In addition, dairy products made from raw goat's milk are widespread, especially in the study area and in Northern Italy in general, with the presence of typical products with protected designation of origin.

Concluding, data obtained in the present study endorsed the possibility for milk to be used in the screening of *T. gondii* infection in goat farms. Furthermore, the analysis of the variation in the amount of specific IgG in serum and milk provided information on the optimal period in which antibody detection on milk should be performed even if the mechanism underlying these changes deserves further investigation. Finally, the detection of *T. gondii* DNA in milk confirmed the potential role of goat milk and raw milk-derived products in human infection by this protozoan; however, further studies are needed to investigate this route of infection.

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Uncited references (please delete the uncited references.)

~~and~~ Koutsoumanis et al. (2018).

Declaration of Competing Interest

None.

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Highlights

- A longitudinal study in a *Toxoplasma gondii* naturally infected goat farm was planned.
- Antibody detection in serum and milk samples and molecular analysis on milk samples.
- An optimal phase of lactation for monitoring *T. gondii* in milk samples was identified.

- During lactation, a discontinuous parasite DNA excretion in milk was recorded.
- The role of caprine raw milk in transmitting the infection has been endorsed.

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