Toxoplasma gondii in raw milk from Sicily

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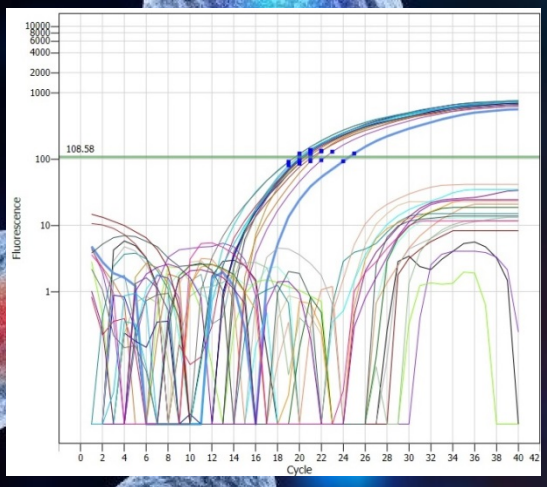
Summary - *Toxoplasma gondii* tachyzoites shed in milk of infected animals are a potential source of human infection, often underestimated. To address the issue, 1381 samples of raw milk from unknown sero-status cows, donkeys, sheep and goats were examined for the detection of *T. gondii* DNA by a real time PCR method targeting the 529 bp repeating element. The protozoan DNA was detected in 67 samples of cow's milk (4,93% and 35% individual and herd prevalence respectively) and in one sample of donkey milk.



Fluorescence curves of the samples examined with real-time PCR for the detection of the 529 bp repeated element of the *T. gondii* genome.

Introduction - According to the existing European legislation (reg. EC 853/2004 and 1662/2006), raw milk from any species can be sold immediately after milking and directly by the producer to the consumer, or to a local milk seller which in turn is the supplier to final consumers, without any thermal treatment except refrigeration between 0 and 4°C (Salimei et al 2010). Although human toxoplasmosis occurs rarely and is mostly linked to consumption of raw milk from infected goats, experimental evidences show that *T. gondii* tachyzoites are able to survive in milk for 3 to 7 days at 4°C (Spišák et al., 2010) and in homemade fresh cheese for a period of 10 days (Hiramoto et al., 2001). Furthermore, the toxoplasmic contamination is worsened due to smaller concentration of proteolytic enzymes that counter the parasite infection in the intestine of children and suckling animals (Ishag et al., 2006).

Materials and methods - 1381 milk specimens were analysed, including 1359 bovine milk samples acquired from 19 different farms, 10 sheep milk, 5 goat milk and 7 donkey milk units. Given that milk contains minor quantities of nucleated cells in comparison to whole blood, prior to DNA extraction, concentration was carried out by centrifugation at 2200 g for 5 minutes. To avoid interference (Mancianti et al. 2013) by casein, 1 ml of pellet was treated with 200 µl TE [1 mM EDTA, 10 mM Tris–HCl (pH = 7.6)] and 300 µl 0.5 M EDTA (pH = 8), then it was resuspended and centrifuged at 3000 g for 10'. In particular, an automatic extraction procedure with magnetic beads (KingFisher Flex Thermo Scientific), and a manual procedure (Nexttec TM DNA isolation systems) were developed. All samples were processed by standardized and validated analytical procedures, referring to the OIE Manual and to the quality system of the accredited laboratory. Samples tested positive for the qualitative Real Time PCR tests, were also examined by quantitative Real Time. Isolation of *T. gondii* strains on Hs27 and /or Bt cell lines was also attempted.

			Esito Real- time PCR qualitativa						
Matrice	Allevamento	Campioni analizzati	Positivi	Negativi	% positività	Tot allevamenti	Positivi	Negativi	% positività
	1	123	0	123	0,00				
	1	41	0	41	0,00				
	1	40	0	40	0,00				
	1	46	0	46	0,00	Bovino 22	7	15	31,82
	1	79	0	79	0,00				
	1	38	0	38	0,00				
	1	136	21	115	15,44	Ovino 5	0	5	0
	1	135	0	135	0,00				
	1	31	0	31	0,00				
	1	36	0	36	0,00				
	1	66	1	65	1,52				
	1	30	1	29	3,33				
	1	85	14	71	16,47	Caprino 5	0	5	0
	1	30	1	29	3,33				
	1	15	1	14	6,67				
	1	80	0	80	0,00	Asina 1	1	0	100
	1	116	28	88	24,14				
	1	67	0	67	0,00				
	2	50	0	50	0,00				
Latte individuale ovino	5	10	0	10	0,00				
Latte individuale caprino	5	5	0	5	0,00				
Latte individuale di asina	1	7	1	6	14,29				

	Campioni analizzati	Positivi	Negativi	% positività
Latte bovino	1359	67	926	4,93
Latte ovino	10	0	10	0,00
Latte caprino	5	0	5	0,00
Latte di asina	7	1	6	14,29
Totale	1381	68	947	4,92

Results and discussion - The protozoan DNA was detected in 67 samples of cow's milk (4,93% and 35% individual and herd prevalence respectively) and in one sample of donkey milk. Allegedly, on farm cats' cohabitation as well as extensive management in the cattle farms that tested positive can be accounted for environmental loading and transmission of *T. gondii* oocysts that are the unique sources of infection for milk-producing animals. Food safety-wise, results are noteworthy since recently raw cow's milk consumption has become increasingly popular with the spread of automatic raw milk vending machines. Albeit heating treatment before consumption is mandatory for consumers, neglect of this requirement cannot be ruled out and could lead the way for foodborne infection. This is particularly true in view of evidences that *T. gondii* tachyzoites in experimentally spiked cow's milk samples could be able to survive in gastric fluids for long enough (1 h) before reaching the intestine and infect the host. Among toxoplasmosis primary infection routes, consumption of tachyzoites-contaminated milk is frequently underestimated. Whether consumption of raw milk from different animal sources can be linked to toxoplasmic infection remains still controversial. Most of the studies agrees on the higher risk of acquiring toxoplasmosis by drinking raw goat milk as compared with the consumption of contaminated raw cow's milk.

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