Supplementary Data 1. Immunohistochemistry protocol.

Immunohistochemistry was performed with the avidin–biotin–peroxidase complex. After deparaffinization and rehydration, antigen retrieval was performed by microwaving slides immersed in citrate buffer. Endogenous peroxidases were inhibited with 3% hydrogen peroxide in methanol for 10 min. Nonspecific immunoreactivity was inhibited with rabbit N-serum diluted 1:50 in 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) incubated for 20 min. Primary antibody, polyclonal rabbit *Toxoplasma gondii* antiserum (catalog 210-70-TOXO; VRMD, Pullman WA) was diluted 1:1000 in 1% BSA-TBS. Slides were incubated with primary antibody and refrigerated overnight. Secondary biotinylated rabbit anti-goat IgG antibody diluted 1:200 in 1% BSA-TBS and slides were incubated for 30 min. Slides were incubated with avidin followed by biotin for 45 min, both diluted in 1:100 phosphate-buffered saline (PBS). The immunohistochemical reaction was developed with 3-amino-9-ethylcarbazole (AEC) chromogen (ImmPact AEC peroxidase substrate; Vector Laboratories, Burlingame, CA) and slides were counterstained with Mayer hematoxylin and mounted in aqueous mounting medium. All washing steps were performed with PBS. For negative controls, TBS and BSA without primary antibody were used; tissue from a cat with confirmed systemic toxoplasmosis served as a positive control. The slides were counterstained with hematoxylin, dehydrated, and mounted under an aqueous mounting medium.

Supplementary Data 2. PCR and sequencing.

All PCR runs were performed in a final reaction volume of 20 µL with the following reagents and primer concentrations: 10 µL of DreamTaq Green PCR master mix (Thermo Fisher Scientific, Waltham, MA), 0.2 µL of BSA (20 mg/mL), 0.4 µg of each primer (see Supplementary Table 1), and 2.8 µL of PCR-grade water. Reactions were performed on a Veriti PCR machine (Applied Biosystems, Thermo Fisher Scientific) with the following cycle conditions: initial denaturation at 95°C for 3 min, followed by 34 cycles of 94°C for 30 s, 60°C for 30 s, and 70°C for 45 s, and a final extension step for 10 min at 72°C. Positive and negative controls were included in each run.

Five µL of PCR product was run on 1% agarose gel with SYBR Safe (Invitrogen, Life Technologies) and visualized with a UV camera (GeneGenius; Syngene, Cambridge, UK). Samples with the correct band size were purified using ExoSAP-IT Express kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions, with slight modifications; samples were incubated on a Veriti PCR machine (Applied Biosystems, Thermo Fisher Scientific) for 10 min at 37°C and 2 min at 80°C. Sanger sequencing was performed by an external company (Macrogen Europe, Amsterdam, The Netherlands) and sequences manually checked and aligned using Geneious (Biomatters, Auckland, New Zealand) before comparison with previously published sequences in GenBank using BLAST.

Supplementary Table 1. PCR primers used in our study of toxoplasmosis in Patagonian maras.

PCR target gene	Primer	Primer sequence	Reference
*CS3** primary	CS3 1F	GTGTATCTCCGAGGGGGTCT	[3]
	CS3 1R	TGTGACTTCTTCGCATCGAC	
*CS3** nested	CS3 2F	AGCGGATTTCCAACACTGTC	
	CS3-R	CTGCTGCATTCACAAACTCC	
*SAG1** primary	SAG1 1F	GTTCTAACCACGCACCCTGAG	[1]
	SAG1 1R	AAGAGTGGGAGGCTCTGTGA	
*SAG1** nested	SAG1 2F	CAATGTGCACCTGTAGGAAGC	
	SAG2 R	GCAAGAGCGAACTTGAACAC	
SAG2 primary	SAG2 1F	GGAACGCGAACAATGAGTTT	[4]
	SAG2 1R	GCACTGTTGTCCAGGGTTTT	
SAG2 nested	SAG2 2F	GAAATGTTTCAGGTTGCTGC	
	SAG2 R	GCAAGAGCGAACTTGAACAC	
GRA6 primary	GRA6 1F	ATTTGTGTTTCCGAGCAGGT	[2]
	GRA6 1R	GCACCTTCGCTTGTGGTT	
GRA6 nested	GRA6 2F	TTTCCGAGCAGGTGACCT	
	GRA6 2R	TCGCCGAAGAGTTGACATAG	

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