Short communication

First identification of *Sarcocystis tenella* (Railliet, 1886) Moulé, 1886 (Protozoa: Apicomplexa) by PCR in naturally infected sheep from Brazil

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1. Introduction

*Sarcocystis* is one of the most prevalent intracellular protozoan parasites in food livestocks such as cattle, sheep and goat. *Sarcocystis tenella* (synonymous = *Sarcocystis ovicanis*) is a pathogenic species transmitted by canids, the definitive hosts (Dubey et al., 1982, 1989a; Tenter, 1995; Adriana et al., 2008). *S. tenella* causes widespread enzootic muscle parasitosis, causing significant losses in the livestock industry due to death of the animal or abortion of pregnant ewes during acute sarcocystiosis, and reduced weight gain, milk and wool production during chronic sarcocystiosis (Dubey et al., 1989a; Pescador et al., 2007) and neurological disease mainly in lambs (Dubey et al., 1989b; Henderson et al., 1997; Yazıcıoğlu and Beyazit, 2005). Sheep become infected with *S. tenella* by ingesting sporocysts with contaminated food or water (Tenter, 1995). Encephalitis may occur in infected sheep, which is similar to two other protozoans, *Toxoplasma gondii* and *Neospora caninum* (Dubey and Beattie, 1988; Dubey, 1990).
In this study, we report two cases of natural infections of *S. tenella* in sheep from Brazil for the first time. Serum test and molecular typing of these sheep samples also suggested co-infection of *T. gondii*.

### 2. Case report

Six hundred and two sheep from Southeast and South regions of Brazil were slaughtered in two different slaughterhouses from São Paulo state, Brazil, and assayed for *T. gondii* infection. None of these animals presented any neurological or respiratory signs at that moment. All of them were initially tested for *T. gondii* antibodies by Indirect Fluorescent Antibody Test (IFAT) (Camargo, 1964) and by Modified Agglutination Test (MAT) (Desmonts and Remington, 1980). The brain and muscle tissue (pool of heart and diaphragm) were bioassayed in mice from serum positive animals, aiming to isolate *T. gondii* strains. Twenty animals presented positive results for serology and bioassay in mice. Two additional sheep born in Brazil, one 2-year-old female Polwarth (Ideal) sheep (#1), a breed from Australia and bred extensively in Santana do Livramento, Rio Grande do Sul State, South region of Brazil; and one 1-year-old male Corriedale sheep (#2), a breed from New Zealand and Australia and bred extensively in Pirajuí, São Paulo State, South-east region of Brazil, resulted positive for serology with higher titers (#1: MAT = 4096, IFAT-IgG = 1024; #2: MAT = 256, IFAT-IgG = 64), but negative for bioassay in mice.

These 22 samples were screened for *T. gondii* infection by Polymerase Chain Reaction (PCR) of the 300-fold repetitive 529 base pair (bp) sequence (Homan et al., 2000). The genotype of *T. gondii* was determined by Restriction Fragment Length Polymorphism (RFLP) using 12 markers including SAG1, 5'-3'SAG2, alt.SAG2, SAG3, BTUB, GRA6, L358, c22-8, c29-6, PK1, Apico and CS3 to genotype *T. gondii* (Su et al., 2006). Six *T. gondii* reference strains (GT1, PTG, CTG, MAS, TgCgCa1 and TgCatBr5) and negative control were included. Twenty sheep were confirmed by genotyping. The typing in 12 markers for sheep #1 and #2 presented products for some markers, as SAG1 (type II or III; #2), 5'-3'SAG2 (type I or II; #2); alt-SAG2 (type II; #2); SAG3 (type III; both animals); Apico (type unique-I; #1), and negative results for other markers. To determine the possibility of co-infection with *S. tenella*, *N. caninum* and Hammondia hammondi in all animals, nested PCR of 18S ribosomal RNA (18S rRNA) was performed using 25 μM of external primers Tg18s48F (5′CCATGCATGTCTAAGTATAAGC3′) and Tg18s359R (5′GTTCACCGTCACTGCCAC3′), and 50 μM of internal primers Tg18s58F (5′CTAAGTATAAGCTTTTATACGGC3′) and Tg18s348R (5′TGCCACGGTAGTCCAATAC3′) (Integrated DNA Technologies, USA) with a 290 base pair (bp) product for *Sarcocystis neurona*, *N. caninum*, *H. hammondi* and *T. gondii*. PCR-RFLP was performed to check the sheep samples with references including *Sarcocystis miescheriana*, *Sarcocystis capracanis*, *Sarcocystis moulei*, *Sarcocystis gigantea*, *Sarcocystis hominis*, *S. tenella*, *S. neurona*, *N. caninum* (Nc-Liv), *H. hammondi* and *T. gondii* (MAS). All cycling reactions were carried out using a MasterCycler EP gradient (Eppendorf, USA), and run in a 1.5% agarose gel electrophoresis to checking the quantity and quality of the PCR products. The products of nested PCR were digested by two sets of restriction enzymes (set 1: AluI and HhaI, to differentiate *S. tenella* from *T. gondii*, *N. caninum* and