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Short communication

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

Rodrigo Costa da Silva [a,*], Chunlei Su [b], Helio Langoni [a]

[a] *Department of Veterinary Hygiene and Public Health, School of Veterinary Medicine and Animal Science, São Paulo State University, 18618-000 Botucatu, SP, Brazil*
[b] *Department of Microbiology, The University of Tennessee, Knoxville, TN 37996, USA*

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ABSTRACT

Sarcocystis tenella is a dog–sheep protozoan parasite, causing a widespread enzootic muscle parasitosis and neurological disease mainly in lambs. This parasite is pathogenic to sheep and important to the economical production of sheep. The present study was initially aimed to determine *Toxoplasma gondii* infection and the occurrence of co-infection with other Apicomplexa parasites in 602 Brazilian sheep. Twenty of these sheep were positive with antibodies to *T. gondii* by MAT and IFAT-IgG tests, positive with PCR-RFLP genotyping at multiple loci, and parasites were isolated from mice infected with sheep tissue samples. Two additional sheep born in Brazil, a 2-year-old female Polwarth (Ideal) sheep, a breed originated from Australia (#1), and a 1-year-old male Corriedale sheep, a breed originated from New Zealand and Australia (#2) were positive to *T. gondii* antibodies by serum tests, and PCR, but negative for bioassay in mice. In genotyping at 12 *loci*, sheep #1 sample and #2 presented positive results only for some markers. PCR-RFLP of 18S ribosomal RNA (18S rRNA) was performed in all 22 animals to identify the possibility of co-infection of *T. gondii* with other Apicomplexa parasites, such as *S. tenella*, *Neospora caninum* and *Hammondia hammondi*, resulting in a *T. gondii* profile for the first 20 animals and a unique genotyping profile for sheep #1 and #2, identical to *S. tenella*. The 18S rRNA PCR products (~310 bp) were sequenced and blasted to GenBank database at NCBI. Both samples were identical to *S. tenella* 18S rRNA gene (GenBank accession number L24383-1). These results suggest the existence of co-infection of *S. tenella* with *T. gondii* in ewes from Brazil.

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 lifestock 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; Yazicioğlu 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).

* Corresponding author at: Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade Estadual Paulista (UNESP), Distrito de Rubião Júnior s/n, 18618-000 Botucatu, SP, Brazil. Tel.: +55 14 38116270; fax: +55 14 38116075.
E-mail address: silva_rcd@yahoo.com.br (R.C. da Silva).

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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′GTTACCCGTCACTGCCAC3′), 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*, and 310 bp for other *Sarcocystis* spp. (Fig. 1). 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: *Alu*I and *Hha*I, to differentiate *S. tenella* from *T. gondii*, *N. caninum* and

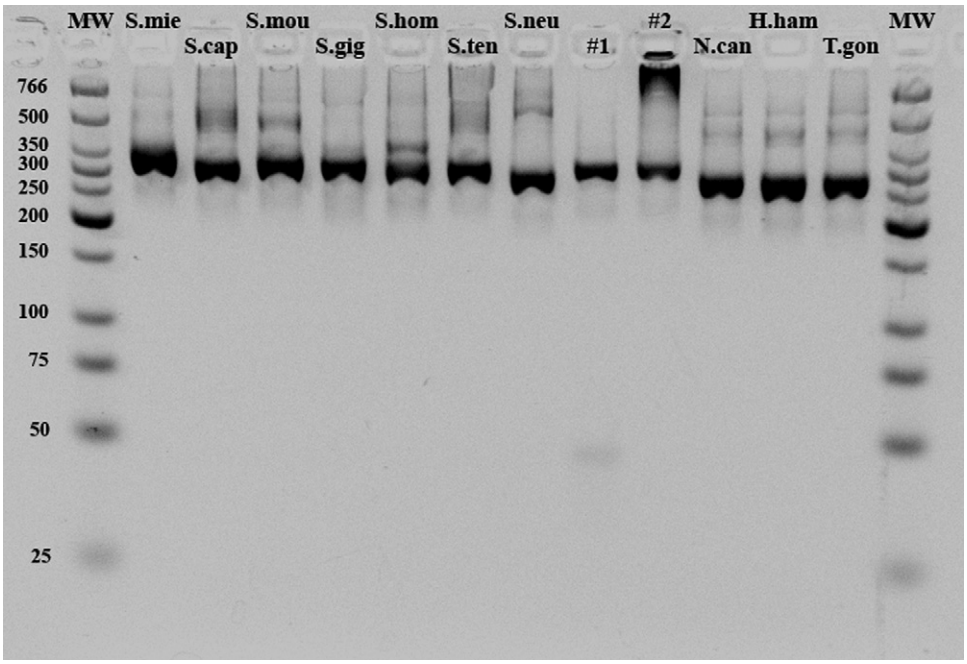

Fig. 1. Nested PCR of 18S rRNA for the reference strains. *Legend*: MW: low molecular weight ladder (New England Biolabs, USA); S.mie: *Sarcocystis miescheriana*; S.cap: *Sarcocystis capracanis*; S.mou: *Sarcocystis moulei*; S.gig: *Sarcocystis gigantea*; S.hom: *Sarcocystis hominis*; S.ten: *Sarcocystis tenella*; S.neu: *Sarcocystis neurona*; #1: Polwarth sheep; #2: Corriedale sheep; N.can: *Neospora caninum*; H.ham: *Hammondia hammondi*; T.gon: *Toxoplasma gondii*.