



Evidence of sexual transmission of *Toxoplasma gondii* in goats



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ABSTRACT

Male goats of reproductive age that were serologically negative for *Toxoplasma gondii* were selected and distributed according to the following arrangement: (A) one goat infected orally with 2.0×10^5 oocysts; (B) one goat infected subcutaneously with 1.0×10^6 tachyzoites; and (C) one uninfected goat kept as a control. After *T. gondii* inoculation, 12 non-pregnant female breeder goats that were serologically negative for the main reproductive diseases, especially toxoplasmosis, were synchronized and then exposed to natural mating by the males that had previously been inoculated: five females exposed to natural mating by male A (group GI); five females exposed to natural mating by male B (group GII); and two females exposed to natural mating by the uninfected male C (group GIII). In serum samples obtained from all the female goats before and after natural mating, the presence of antibodies against *T. gondii* was investigated using the ELISA test. PCR was performed on semen samples, on females and fetal tissues and placenta. Ten out of the 12 females showed specific antibodies against *T. gondii* after natural mating: five in GI and five in GII. On several dates on which natural mating occurred, *T. gondii* was identified in semen samples from the infected males, using PCR. Subsequently, after the females had been sacrificed, it was also possible to identify *T. gondii* in tissue samples from the infected females and from their fetuses, stillbirths and offspring, using PCR. Therefore, these results prove, for the first time, that *T. gondii* infection can be transmitted sexually from male to female goats.

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1. Introduction

Toxoplasma gondii is a protozoon with a facultative heteroxenous life cycle that can infect all warm-blooded animal species, including mammals, birds and humans. Toxoplasmosis is a zoonosis of cosmopolitan character and medical and veterinary importance, accounting for abor-

tions and congenital disease in several intermediate host species (Tenter et al., 2000).

Goats are economically important in Brazil, with an estimated herd of more than nine million animals (IBGE, 2013). They are an important source of meat and milk for humans. Because of their economic importance and the public health importance of toxoplasmosis infection, epidemiological studies on toxoplasmosis in goats have been conducted in Brazil. The prevalence of toxoplasmosis in Brazil ranges from 10 to 92.4%, according to the geographical region and diagnostic technique used (Garcia et al., 2012).

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Although *T. gondii* has been successfully isolated from the semen of sheep (Spence et al., 1978; Lopes et al., 2009), goats (Dubey and Sharma, 1980; Santana et al., 2010), cattle (Scarpelli et al., 2009) and pigs (Moura et al., 2007), there are no studies in the literature demonstrating whether sexual transmission of this coccidian is viable in goats. Thus, the present study aimed to evaluate the feasibility of sexual transmission of *T. gondii* in female goats that were naturally covered by males that had been experimentally infected with *T. gondii*.

2. Materials and methods

2.1. Male goats

Three male goats of undefined breed, aged between one and two years, were selected. They were certified to be serologically negative for brucellosis according to the acidified buffered antigen test (Alton et al., 1988), leptospirosis according to the microscopic agglutination test (Cole et al., 1973) and neosporosis and toxoplasmosis according to the indirect immunofluorescence (IFI) test (Camargo, 1964).

The *T. gondii* strains used in the present study were “P” (Jamra and Vieira, 1991) and “RH” (Sabin, 1941). These strains are maintained at the Animal Health Research Center (CPPAR), UNESP, Jaboticabal, Brazil. The “P” and “RH” strains have been genotypically characterized and classified as Type III (Bresciani et al., 2009), and Type I (Pena et al., 2008; Herrmann et al., 2010), respectively.

The three animals were identified and randomized as follows: (A) orally infected with 2×10^5 P strain oocysts ($n = 1$); (B) subcutaneously infected with 1×10^6 RH strain tachyzoites ($n = 1$); and (C) non-inoculated control ($n = 1$). To confirm that *T. gondii* infection was present in the serum of all the breeding male goats, samples were obtained on days –14 and –1 before infection (BI) and on days 1, 3, 5, 7, 9, 11, 14, 21, 28, 35, 42, 49, 56, 63 and 70 post-infection (PI), and were assayed for antibodies against *T. gondii* using IIF (Camargo, 1964). The reaction was considered positive when the titers were greater than or equal to 1:64.

Ejaculates from the three experimentally infected goats were collected, using an electro-ejaculatory method, on days –1 BI and 1, 3, 5, 7, 9, 11 and 14 PI. The methods proposed by Fuentes et al. (1996) were used for semen sample standardization. DNA samples extracted from *T. gondii* were used as the positive control and the investigations on the other samples (semen from male goats and tissue samples from female goats and their fetuses, stillbirths and offspring) were performed using a DNA kit (QIAamp DNA Mini Kit, Qiagen, USA), following the manufacturer's recommendations.

2.2. Female goats

For this step, 12 crossbred goats (Boer $\times$ Saanen) were selected. They were 12–18 months of age, not pregnant and serologically negative for reproductive diseases according to the aforementioned methods. Subsequently, these females were randomly assigned to the males.

The dates on which the females were covered by the males that had been experimentally infected with *T. gondii* were chosen based on the findings of Santana et al. (2010). These authors used bioassays and PCR to diagnose the post-inoculation days on which males goats shed this parasite in semen. Thus, five female goats (group GI) were covered naturally on days 21, 28, 42, 56 and 70 PI (one on each day) by the male infected with 2×10^5 oocysts of *T. gondii* (A); five (group GII) were covered on days 7, 21, 28, 49 and 70 PI by the male infected with 1×10^6 tachyzoites of *T. gondii* (B); and the remaining two females (group GIII) were covered naturally on random dates by the uninfected male (C).

For estrus in breeding goats to be manifested on pre-established dates, a short protocol for synchronization of estrus in small ruminants was used, as described by Maia (1998).

Serum samples were collected from the female goats on days –14, –7 and –1 before natural coverage (BNC) and on days 3, 6, 9, 11 and 14, and weekly thereafter until the end of the gestational period, after natural coverage (ANC). These were investigated for the presence of antibodies to *T. gondii* using ELISA test. For this, a suspension of tachyzoites (10^7 – 10^8 parasites/ml) was prepared and subjected to 11 cycles of freezing at -70°C and thawing at 35°C in a water bath. After the final thawing, the samples

were kept in an ice bath and sonicated for three cycles, for one to two seconds. The concentration of soluble protein antigen used in the ELISA test was determined using the Hartree (1972) method.

To achieve the optimal dose of antigen reactivity, the serum and conjugate were standardized using the optimal antigen dilution (concentration of $5\text{ }\mu\text{g/mL}$) in carbonate–bicarbonate buffer (0.05 M ; pH 9.6), and the reference positive and negative serum at a dilution of 1/200 in 0.01 M PBS buffer (pH 7.4), containing 0.05% Tween 20 (PBS–Tween) plus 5% normal rabbit serum. The conjugate used consisted of monoclonal anti-IgG from goats and sheep coupled with alkaline phosphatase and diluted 1/50,000 in PBS–Tween plus 5% normal rabbit serum.

The positive reference serum used was a blood sample taken on day 28 PI from the male goat inoculated with oocysts, with seropositivity proven by both IIF and Elisa. The negative reference consisted of blood collected from the same goat as sampled before the experiment, and was demonstrated to be negative with the same techniques.

The reaction was measured using an ELISA reader with a 405 nm filter. The “white” of the reaction was taken to be the microplate well that contained all the elements of the reaction except the serum from the female goats. The absorbance values of serum samples tested were compared with the average values found for the reference serum sample that was negative for *T. gondii*.

Immediately after birth took place, or any abnormal events such as abortions or premature birth, the female goats were ethically sacrificed in order to sample the following tissues: skeletal and cardiac muscle, brain/cerebellum, spleen, liver, uterus, vagina and ovary. Placenta and fetal organs such as brain, skeletal muscle, liver and spleen were also sampled. All of these tissues as well as semen samples were screened for genetic material of *T. gondii* using PCR (Fuentes et al., 1996). This study was approved by the Animal Experimentation Ethics Committee of the School of Agricultural and Animal Sciences, São Paulo State University, under protocol no. 010850-08.

3. Results and discussion

3.1. Males

The infected state of the male goats was confirmed by the seroconversion of the inoculated animals. In the males inoculated with oocysts and tachyzoites, seroconversion occurred on days 28 and 14 PI, respectively. The animal kept as a control did not show any antibodies against the parasite.

In this study, on the semen samples assessed for genetic material (DNA) of *T. gondii* by PCR, was observed to be present in some of the semen samples from the infected male goats (A and B) and it was absent in the uninfected male (C).

The results obtained by Santana et al. (2010) among goats inoculated with oocysts and tachyzoites of *T. gondii* were similar to those of the present study. These authors confirmed that seroconversion of the inoculated animals had occurred and identified the protozoon at various dates through bioassays in mice, as well as using PCR.

3.2. Females

Among the twelve female goats, three that had been covered by the male inoculated with tachyzoites had gestational complications: two episodes of abortion and one birth of two premature offspring that died after 24 h. The abortions were possibly related to an outbreak of parasitemia. These findings are consistent with those of Moraes et al. (2010), who reported occurrences of stillbirth and early embryonic death associated with toxoplasmosis. The

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