Parasitology International 63 (2014) 865–867

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Toxoplasma gondii and *Neospora caninum* infections in goat abortions from Argentina

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ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 4 July 2014
Accepted 25 July 2014
Available online 14 August 2014

Keywords:
Toxoplasma gondii
Neospora caninum
Goat
Abortion

ABSTRACT

The aims of this study were to identify the occurrence of *Toxoplasma gondii* and *Neospora caninum* abortions in goats from Argentina by serological, macroscopical and microscopical examination and bioassay, and to characterize the obtained isolates by molecular techniques. For this purpose, 25 caprine fetal fluids, 18 caprine fetal brains and 10 caprine placentas from 8 dairy/meat goat farms from Argentina were analyzed. Gestational age of the aborted fetuses was determined in 18 cases. Protozoal infections were detected by at least one of the applied diagnostic techniques in 44% (11/25) of examined fetuses; specifically, 24% (6/25) were positive to *T. gondii*, 8% (2/25) were positive to *N. caninum* and 12% (3/25) were positive to both parasites. In this study IFAT titers were similarly distributed in younger and older fetuses. Macroscopical and microscopical examination of one placenta revealed chalky nodules in the fetal cotyledons and normal intercotyledonary areas, as well as necrosis and calcification of mesenchymal cells in villi. Tachyzoites were observed in peritoneal wash from 2 mice inoculated with brain and a pool of brain and placenta of two fetuses. Cell culture growth of tachyzoites was achieved from one inoculated mouse, and confirmed as *T. gondii* by PCR. The *T. gondii* isolate was identified as atypical or non-canonical by nested-PCR-RFLP. This is the first study that investigated the involvement of *N. caninum* and *T. gondii* in cases of goat abortion in Argentina.

1. Introduction

Toxoplasmosis is an important cause of caprine abortions and the ingestion of undercooked meat and unpasteurized milk from infected goats could be the source of infection for humans [1]. Neosporosis infection in sheep and goats is uncommon and only a few cases of abortion or congenital disease have been reported [2]. *Toxoplasma gondii* abortion in sheep and goats is frequently associated with necrosis and calcification of the fetal cotyledons and normal intercotyledonary areas [1] and similar lesions can be also observed in *Neospora caninum*-associated abortion [1,2].

In Argentina, antibody detection for *T. gondii* and *N. caninum* in goats has been reported [3] but no data are available about goat abortion due to these parasites. The aims of this study were (i) to identify the occurrence of *T. gondii* and *N. caninum* abortions in goats from Argentina by serological, macroscopical and microscopical examination and bioassay, and (ii) to characterize the obtained isolates by molecular techniques.

2. Materials and methods

2.1. Samples

Caprine fetal fluids ($n = 25$), caprine fetal brains ($n = 18$) and caprine placentas ($n = 10$) were analyzed to diagnose *T. gondii* and *N. caninum* infections at the Laboratory of Immunoparasitology, FCV, UNLP. At necropsy, fetal body fluids were aspirated from the thoracic cavity and brain and/or placental cotyledons were collected. Samples were obtained from 8 dairy/meat goat farms from Argentina that tested negative for brucellosis. Gestational age of the aborted fetuses was determined in 18 cases. Of these, 1 was less than 60 days old (f12), 4 were aborted in the midterm of pregnancy (60–120 days old, f16–19) and 13 were aborted at late pregnancy (more than 120 days old, f1–f11, f13 and f14). The gestational age of the remaining 7 fetuses was unavailable (f15, f20–f25).

2.2. Serological diagnosis

Fetal fluid samples were examined by indirect fluorescence antibody test (IFAT) for specific antibodies to *T. gondii* and *N. caninum* as described previously [4]. Samples were diluted twofold in PBS from 1:25 or fourfold from 1:16 dilution.

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http://dx.doi.org/10.1016/j.parint.2014.07.009
1383-5769/

2.3. Bioassay in mice/gerbils and cell culture

Brain samples from f1, f2, f3, f4, f16, f17, f18, and f19 and a pool of brain and placenta of f5, f6, f7, f8, f9, f10, f11, f12, f13, and f14 were homogenized and digested with pepsin and HCl as described by Dubey [5]. Samples f1, f2, f3, f4, f5, f6, f7, f8 and f9 were inoculated intraperitoneally into two N:NIH Swiss mice for *T. gondii* isolation. Samples f10, f11, f12, f13, f14, f15, f16, f17, f18 and f19 were inoculated intraperitoneally into two N:NIH Swiss mice and two Mongolian gerbils (*Meriones unguiculatus*) for *T. gondii* and *N. caninum* isolation, respectively. Mortality was recorded daily for 30 days after inoculation. Mice and gerbils were bled 30 days post-inoculation (dpi) and serum samples were examined by IFAT as described previously [6]. When clinical signs were observed, inoculated animals were euthanized and the peritoneal cavity was washed with saline. Peritoneal wash was inoculated into Vero cell cultures and supernatants containing tachyzoites were collected and kept at −20 °C until DNA extraction [6]. Animal handling and all experimental procedures were carried out in compliance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Research Council (Academy Press, 1996, Washington, USA)

2.4. Macroscopical and microscopical examination of placentas

Each placenta was immersed in isotonic saline solution to detect macroscopical lesions. Formalin-fixed samples of placentas were routinely processed for histology and stained with hematoxylin and eosin (H&E).

2.5. PCR diagnosis and genotyping of T. gondii

DNA was extracted from tachyzoites isolated from cell cultures using the Wizard® Genomics DNA Purification kit (Promega, USA). Specific PCR techniques using B22–B23 and TOX4–TOX5 primers and NP6 + −NP21 + were used to identify *T. gondii* and *N. caninum* DNA respectively, as described previously [7].

Toxoplasma gondii genotyping was carried out by multilocus RFLP using the PCR-amplified markers nSAG2, SAG3, BTUB, GRA6, c22-8, L358, PK1, c29-2 and Apico as described previously, with RH [8], Me49 [9] and NED as *T. gondii* reference strains [7].

3. Results

Upon initial examination, f1 was macerated and fetuses f2, f3, f4, f5, f6, f7, f8, f9, f10, f11, f12, f13, f14, f16, f17, f18 and f19 appeared normal. Fetus 12 was 9 cm of length and mummified. Fetuses 13 and f14 were twins with severe signs of autolysis.

Protozoal infections were detected by at least one of the applied diagnostic techniques in 44% (11/25) of examined fetuses; specifically, 24% (6/25) were positive to *T. gondii*, 8% (2/25) were positive to *N. caninum* and 12% (3/25) were positive to both parasites. Results of all techniques used for each positive fetus are summarized in Table 1. In addition, the distribution of positive fetuses to *T. gondii* and *N. caninum* by IFAT and bioassay diagnostic techniques according to gestational age is summarized in Table 2. From the 8 studied farms 12.5% (1/8) were positive to *T. gondii*, 12.5% (1/8) were positive to *N. caninum*, 25% (2/8) were positive to both parasites and 50% (4/8) were negative.

Tachyzoites were observed in peritoneal wash from mice inoculated with brain of f2 and with brain and placenta of f13. *Toxoplasma gondii* specific antibodies (IgG ≥1024) were detected in these mice. Neither *T. gondii* nor *N. caninum* specific antibodies were identified in mice inoculated with tissues from other fetuses.

Cell culture growth of tachyzoites was achieved only from mice inoculated with f2 and confirmed as *T. gondii* by PCR. Although parasite cell culture isolation was not accomplished from f13, macroscopical and microscopical examination of the placenta revealed chalky nodules in the fetal cotyledons and normal intercotyledonary areas, as well as necrosis and calcification of mesenchymal cells in villi.

Table 1
Toxoplasma gondii and *Neospora caninum* positive fetuses by different techniques.

Fetus	*T. gondii* IFAT	Bioassay in mice	*N. caninum* IFAT	Bioassay in gerbils
F2	NEG	+	NEG	NEG
F3	16	NEG	NEG	NEG
F5	16	NEG	NEG	NEG
F6	16	NEG	NEG	NEG
F10	NEG	NEG	800	NEG
F11	12,800	NEG	NEG	NEG
F13	4096	+	1600	NEG
F14	4096	NEG	1600	NEG
F18	1024	NEG	NEG	NEG
F19	64	NEG	16	NEG
F25	NEG	ND	800	ND

(+): positive.
NEG: negative.
ND: not done.

The *T. gondii* isolate from f2 was identified as atypical or non-canonical by nested-PCR-RFLP, with most markers showing a type III restriction pattern, except BTUB (type II) and L358 (type I).

4. Discussion

In the present work, we used immunoserological diagnosis, macroscopical and microscopical examination of placentas, bioassay in mice/gerbil and cell culture to detect the presence of protozoal infection in aborted fetuses from Argentinean goats. To our knowledge this is the first study that investigated the involvement of *N. caninum* and *T. gondii* in cases of goat abortion in Argentina.

Protozoal infection was present in 44% of examined fetuses, based on at least one of the applied diagnostic techniques. Thirty six percent (9/25) were positive for *T. gondii* infection by serology and/or bioassay, while 20% (5/25) were positive to *N. caninum* only by serology. Three infected fetuses were serologically positive to both parasites. Usually, diagnosis of abortion etiology is difficult for veterinarians working with small ruminants [10]. Considering the type of placenta in goats, detection of antibodies in fetal fluids indicates transplacental infection [11]. Gestational age is an important factor to analyze because small ruminant fetuses are immunocompetent since 50 days of gestation [12], and younger fetuses are unable to generate antibodies, remaining serologically negative. In the present study, gestational age was unavailable in 7/25 fetuses; therefore, it is possible that some of these fetuses were infected with *T. gondii* or *N. caninum* but were unable to mount an immune response due to low gestational age. If this were the case, the real seroprevalence of protozoal infections could be underestimated.

Toxoplasma gondii infection was confirmed in 36% of aborted fetuses by several techniques, and in agreement with Pereira-Bueno et al. [13], the detection of fetal *T. gondii* infection varied according to the diagnostic technique used. In our study, more positive fetuses were observed by serology than by histopathology of the placenta. Pereira-Bueno et al. [13] reported that IFAT titers ≥128 were predominant in younger fetuses (60–120 days gestational age) and lower titers in aborted fetuses during the last month of gestation (>120 days). In this study IFAT titers were similarly distributed in younger and older fetuses.

We observed characteristic lesions of protozoal infection by macroscopical examination and histopathology in 1/7 examined placentas. This data is lower than reported in previous studies elsewhere [13,14], probably due to the smaller number of placentas analyzed in our study. Considering all these facts, it is possible to suggest that toxoplasmosis is an important cause of abortion in Argentinean goats as stated in other countries [1,13,15]. However, since macroscopical and/or microscopical lesions in fetuses with low IFAT titers were not analyzed, other causes of fetal death cannot be ruled out.

Table 2
Number and percentage of positive fetuses to *Toxoplasma gondii* and *Neospora caninum* by IFAT and bioassay diagnostic techniques according to gestational age.

T. gondii				*N. caninum*		
Fetal age (days)	Serology	Bioassay	Total	Serology	Bioassay	Total
<60	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
60–120	2/4 (50)	0/4 (0)	2/4 (50)	1/4 (25)	0/4 (0)	1/4 (25)
>120	6/13 (46.1)	2/13 (15.4)	8/13 (76.9)	3/13 (23)	0/13 (0)	3/13 (23)
Unknown	0/7 (0)	0/7 (0)	0/7 (0)	1/7 (14.2)	0/7 (0)	1/7 (14.2)
Total	8/25 (32)	2/25 (8)	10/25 (40)	5/25 (20)	0/25 (0)	5/25 (20)

Percentage in parenthesis.

Neospora caninum infection was detected only by fetal fluid serology, providing evidence of transplacental infection and suggesting *N. caninum* as a potential cause of abortion in goats, as mentioned by others [15]. *Neospora caninum* was found in 1 of 12 aborted fetuses in Spain by histological and immunohistochemical techniques [15]. Our results showed that 20% of aborted fetuses presented *N. caninum* infection. These differences may be influenced by the different methodologies employed for diagnosis or management practices such as the higher number of dogs present in Argentinean farms. However, further studies are necessary to investigate the role of *N. caninum* as a cause of abortion in goats and to evaluate the economic losses for the Argentinean farmers.

Interestingly, mixed infection with *N. caninum* and *T. gondii* was recorded in 3 fetuses. Two of them were twins and antibody titers against both parasites were high (*T. gondii* IFAT 4096 and *N. caninum* IFAT 1600). From one of the fetuses (f13) *T. gondii* was isolated; however the cause of abortion remains uncertain. A possible synergic effect could be produced by both parasites. More studies are necessary to confirm this hypothesis.

The *T. gondii* genotype isolated from f2 is considered atypical or non-canonical. This finding is in agreement with other reports about *T. gondii* genotyping from animals in Argentina and Brazil [16,17]. These atypical genotypes, apparently more frequent in South America than in other parts of the world, could be explained, at least in part, to a higher recombination rate that took place in the intestine of felids [18]. The role and virulence of these particular genotypes on transplacental infection and/or caprine abortion should be further investigated.

Acknowledgments

We gratefully acknowledge the technical support of Isidoro Ercoli.

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