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High prevalence and genotypes of *Toxoplasma gondii* isolated from goats, from a retail meat store, destined for human consumption in the USA

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ABSTRACT

Little information is available concerning the presence of viable *Toxoplasma gondii* in tissues of goats worldwide. In the present study, hearts of 234 goats obtained from a local USA grocery store were examined for *T. gondii* infection. Blood clot or fluid removed from each heart was tested for antibodies to *T. gondii* by using the modified agglutination test (MAT). Antibodies to *T. gondii* were found in 125 (53.4%) of 234 goats, with titers of 1:5 in 20, 1:10 in 44, 1:20 in 16, 1:40 in five, 1:160 in five, 1:320 in five, and 1:640 or higher in 30 goats. Hearts of 112 goats (46 goats <1:5, and 66 goats 1:10 or higher) were used for isolation of viable *T. gondii* by bioassays in mice. For bioassays, 50 g of the myocardium were digested in an acid pepsin solution and the digest inoculated into mice; the recipient mice were examined for *T. gondii* infection. *Toxoplasma gondii* was isolated from 29 goats; from hearts of one of 46 with titers of <1:5, one of nine with titers of 1:10, one of three with titers of 1:40, and 26 of 40 with titers of 1:160 or higher. Two isolates were highly virulent to outbred Swiss Webster mice; all infected mice died of toxoplasmosis, irrespective of the dose. All *T. gondii* isolates were subsequently grown in cell cultures. Genotyping of the 29 *T. gondii* isolates using 10 PCR-restriction fragment length polymorphism markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico) from DNA obtained from cell culture grown tachyzoites revealed 12 genotypes. Nine isolates were clonal Type II lineage, four isolates had type II alleles at all loci except a type I allele at the Apico locus, and four isolates were clonal Type III. The remaining 12 strains were divided into nine atypical genotypes, including five new and four previously identified genotypes. DNA sequences of four introns (EF1, HP2, UPRT1 and UPRT7) and two genes (GRA6 and GRA7) were generated for the five new genotypes. Comparing these sequences with previously published data revealed no unique sequences in these goat strains. Taken together, these results indicate high parasite prevalence and moderate genetic diversity of *T. gondii* in goats, which have important implications in public health. We believe this is the first genetic analysis of *T. gondii* isolates from goats in the USA.

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1. Introduction

Toxoplasma gondii infects virtually all warm-blooded animals including humans, livestock and marine mammals (Dubey, 2009). In the USA, various surveys have found that 10–50% of the adult population has been exposed to this parasite (Dubey and Beattie, 1988; Dubey, 2009; Jones et al., 2001, 2003, 2007). *Toxoplasma gondii* infection causes mental retardation, loss of vision and other congenital health problems in human infants. Toxoplasmosis is an important cause of morbidity and mortality in immunosuppressed individuals and can cause serious health problems in healthy adults (Luft et al., 1993; Montoya and Liesenfeld, 2004). *Toxoplasma gondii* is one of three pathogens (together with *Salmonella* and *Listeria*) which account for >75% of all deaths due to foodborne disease in the USA (Mead et al., 1999).

Humans become infected post-natally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. It is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to

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the parasite strain, host variability or other factors. Recently, attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts (Howe et al., 1997; Grigg et al., 2001). Severe cases of toxoplasmosis have been reported in immunocompetent patients, considered to be due to infection with atypical *T. gondii* genotypes (Ajzenberg et al., 2004; Demar et al., 2007; Elbez-Rubinstein et al., 2009; Grigg and Sundar, 2009).

The proportion of the human population that acquires infection by ingestion of oocysts in the environment or by eating contaminated meat is not known and currently there are no tests available that can determine the infection source. In the USA, poultry, pork and beef are the main meat types consumed. In a nationwide study of the prevalence of *T. gondii* in retail meat, viable *T. gondii* was isolated from seven of 2094 pork samples but not from 2094 beef or 2094 chicken meat samples (Dubey et al., 2005). Thus, while the scope of human infection resulting from meat sources remains undetermined, the low prevalence of *T. gondii* infection in market pigs would not account for the seroprevalence in humans in the USA.

Toxoplasmosis continues to be a public health problem in the USA. Annually, an estimated 1,075,242 persons are infected with *T. gondii* and approximately 4.839 persons develop symptomatic ocular disease each year (Jones and Holland, 2010). A recent study identified drinking unpasteurised goat milk as a risk factor for recently-acquired toxoplasmosis in pregnant women in the USA (Jones et al., 2009). Feeding of goat whey was also identified as a source of *T. gondii* infection in pigs in the Netherlands (Meersburg et al., 2006). Clinical and even fatal toxoplasmosis has been reported in humans after drinking goat milk (Riemann et al., 1975; Patton et al., 1990; Skinner et al., 1990). Although pasteurisation will kill *T. gondii* in milk, unpasteurised, raw milk is sold by small goat farmers and goat cheeses made from raw milk could be a source of *T. gondii* infection. Little is known of the excretion of *T. gondii* in goat milk (Dubey, 2009). There are no recent surveys for the prevalence of *T. gondii* infection in goats in the USA. Goat meat is also very popular with many ethnic groups in the USA. In the present study, we determined the prevalence of *T. gondii* infection in goat meat destined for human consumption and genetically characterised the isolates.

2. Materials and methods

2.1. Naturally-infected goats

Between October 2009 and May 2010, hearts of 234 goats from a local retail meat store in Beltsville, Maryland, USA were obtained for the present study. The goats were thought to be raised as small flocks in Maryland, Virginia and Pennsylvania, and were between six and 12 months old. No other information could be gathered for these animals. The heart of each goat was placed in a ziplock bag and transported to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland, USA. Blood clot or fluid from the heart was removed, centrifuged and serum was separated. One to 4 days elapsed between killing of goats and processing of hearts for *T. gondii* examination. The heart was selected for bioassays based on convenience and because one can obtain blood clot or fluid from inside the heart for serological examination.

2.2. Serological examination

Sera of goats were tested for *T. gondii* antibodies with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). The antigen was prepared at the Laboratory of Parasitology, National Reference Centre on Toxoplasmosis (Reims, France), as described by Desmonts and Remington (1980). Sera were diluted twofold from 1:5 to 1: 640 or higher (Table 1).

2.3. Bioassay of goats for T. gondii infection

Hearts of 112 goats were bioassayed for *T. gondii* in mice (Table 1). After removing fat, auricles and blood, 50 g of the myocardium from each heart were chopped and gently ground in a blender without any fluid. One hundred ml of aqueous 0.85% NaCl solution (saline) were then poured into the blender and the remaining heart tissue was homogenised for 30 s at top speed. This homogenate was incubated with an acid pepsin solution for 1 h at 37 °C, centrifuged, the sediment neutralised and suspended in 5–10 ml antibiotic solution (Dubey, 2009). The homogenate was inoculated (1 ml/mouse) s.c. into one or two IFNγ gene knockout (KO) mice and three or four female Swiss Webster (SW) or BALB/c mice; totalling five mice per heart (Dubey et al., 2008b; Table 2).

Inoculated mice were examined for *T. gondii* infection. Tissue imprints of lungs and brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 41 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 43 days p.i. and brains of all mice were examined for tissue cysts as described (Dubey, 2009). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.4. Mouse virulence of the caprine T. gondii isolates

Pathogenicity of tachyzoites and oocysts of three isolates (TgGoatUs4, TgGoatUs6, TgGoatUs26) were compared in SW mice (Table 3). Oocysts were obtained by feeding infected tissues of mice (see Section 3) to cats (Dubey et al., 2008b). Oocysts were counted and diluted 10-fold from 10^{-1} to 10^{-7} to reach an end point of ≅1 oocyst. Aliquots (0.5 ml) from each dilution were orally inoculated into five mice. To obtain tachyzoites, cell cultures were seeded with homogenates of mesenteric lymph nodes from mice orally inoculated with oocysts. Individual tachyzoites were obtained by passing the infected cell cultures through a 27 gauge needle and a 3 μm filter (Nuceopore, Whatman Inc., Clifton, NJ, USA). Tachyzoites were counted in a hemacytometer and diluted 10-fold serially in saline to ensure that the last two dilutions did not have a tachyzoite. Aliquots from each were inoculated s.c. into five SW mice. The last infective dilution was considered to have one viable organism for data presentation in Table 3.

Additionally, virulence of oocysts of two more isolates (TgGoatUS2, Type II and TgGoatUS13, atypical) were compared in SW mice.

Table 1
Serological and parasitic prevalence of *Toxoplasma gondii* in goats from a local retail meat store in Maryland, USA.

MAT titer[a]	No. of goats	No. of bioassayed	No. of *T. gondii* isolates
<5	109	46	1
5	20	0	0
10	44	9	1
20	16	14	0
40	5	3	1
160	5	5	3
320	5	5	2
⩾640	30	30	21
Total	234	112	29

[a] MAT, modified agglutination test.

Table 2
Isolation of *Toxoplasma gondii* from hearts of goats from a retail meat store in Maryland, USA.

Goat No.	Date	MAT titer	Bioassay in mice[a]		Isolate designation
			(BALB/c[c] or SW[d])	KO	
24	10/13/2009	⩾640	3/3[c](2 PCR positive but cysts not seen)	2/2 (21,21)[b]	TgGoatUS1
26	10/13/2009	⩾640	3/3[c](3 PCR positive but cysts not seen)	2/2[b] (19,19)	TgGoatUS2
27	10/15/2009	⩾640	3/3[c]	2/2[b] (17,17)	**TgGoatUS3**[e]
29	10/15/2009	10	1/3[c]	0/2[b]	TgGoatUS4
34	10/20/2009	⩾640	3/3[c] (34)	2/2[b] (17,25)	**TgGoatUS5**[e]
41	10/22/2009	⩾640	3/3[c] (18,19,27)	2/2[b] (16,16)	TgGoatUS6
42	10/22/2009	⩾640	0/3[c]	1/2[b] (23)	TgGoatUS7
43	10/22/2009	⩾640	2/3[c]	1/2[b] (18)	**TgGoatUS8**[e]
46	10/22/2009	⩾640	0/3[c]	1/2[b] (21)	TgGoatUS9
51	10/27/2009	⩾640	1/3[c]	2/2[b] (45,48)	TgGoatUS10
56	10/29/2009	⩾640	1/3[d]	1/1[b] (24)	TgGoatUS11
63	11/16/2009	<5	0/4[d]	1/1[b] (17)	TgGoatUS12
71	11/19/2009	⩾640	2/4[d]	1/1[b] (21)	TgGoatUS13
80	12/3/2009	⩾640	3/4[d]	1/1[b] (13)	TgGoatUS14
81	12/3/2009	⩾640	4/4[d]	1/1[b] (19)	TgGoatUS15
88	12/3/2009	⩾640	1/4[d]	1/1[b] (20)	TgGoatUS16
135	1/21/2010	⩾640	2/3[d]	1/2[b] (43)	TgGoatUS17
148	1/28/2010	320	1/4[d]	1/1[b] (25)	TgGoatUS18
166	3/8/2010	320	0/3[d]	2/2[b] (15,15)	TgGoatUS19
176	3/11/2010	⩾640	0/3[d]	1/2[b] (13)	**TgGoatUS20**[e]
181	3/16/2010	40	1/3[d]	0/2[b]	TgGoatUS21
190	1/4/2010	160	3/3[d]	2/2[b] (20,25)	TgGoatUS22
194	1/4/2010	320	2/3[d]	1/2[b] (13)	TgGoatUS23
196	1/4/2010	⩾640	2/3[d]	1/2[b] (35)	TgGoatUS24
201	1/4/2010	⩾640	0/3[d]	2/2[b] (18,24)	TgGoatUS25
205	1/4/2010	⩾640	1/3 [d](23)	0/2[b]	**TgGoatUS26**[e]
209	4/6/2010	⩾640	2/3[d]	1/2[b] (19)	TgGoatUS27
223	4/27/2010	160	3/3[d]	2/2[b] (16,19)	TgGoatUS28
230	5/4/2010	160	1/3	0/2	TgGoatUS29

MAT, modified agglutination test.
[a] No. of mice *T. gondii* positive/No. of mice inoculated.
[b] No. of mice dead. Day of death is in parentheses.
[c] BALB/c mice.
[d] Swiss Webster (SW) mice.
[e] Bold designates new genotypes.

Table 3
Infectivity of oocysts and tachyzoites of three *Toxoplasma gondii* isolates from goats to Swiss Webster mice.[a]

Dose[b]	TgGoatUS6 (goat 41, cat 66)		TgGoatUS4 (goat 29, cat 70)		TgGoatUS26 (goat 205, cat 76)		
	Oocysts (oral)	Tachyzoites (s.c.)	Oocysts (oral)	Tachyzoites (s.c.)	Oocysts (oral)	Oocysts (s.c)	Tachyzoites (s.c.)
100,000	5[a] (6 or7)	ND	5 (4)	ND[d]	5 (4–7)	5 (5–9)	ND
10,000	5 (4 or 5)	ND	5 (5–7)	ND	5 (7)	5 (8–10)	5 (16–19)
1,000	5 (6 or 7)	5 (13–17)	5 (7 or 8)	5 (survived)	5(8 or 9)	5 (10 or 11)	5 (21 or 22)
100	5 (8 or 9)	5 (14–45)	5 (10,13, 3 survived)	5 (survived)	5 (9–11)	5 (11–14)	5 (22 or 23)
10	5 (9–14)	5 (24–32,68[e])	5 (survived)	5 (survived)	5 (10–15)	5 (16–20)	2 (34,37)
1[b]	4 (10–21)	2 (24,26)	3 (survived)	2 (survived)	3 (13–17)	2 (23,25)	1 (34)
<1	0[c]	0[c]	0[c]	0[c]	0[c]	0[c]	0[c]

[a] Five mice were inoculated in each group. Day of death (range) is indicated in parentheses.
[b] Estimate based on one organism being an infective dose.
[c] None of the five mice became infected; no *T. gondii* antibody and no tissue cysts.
[d] ND, not done.
[e] Sick mouse euthanised and many tissue cysts found in its brain.

2.5. Animal ethics approval

All mice and cats used in experiments were handled using procedures approved by the Animal Care Program, U. S. Department of Agriculture, USA.

2.6. Genetic characterisation of goat isolates of T. gondii

Toxoplasma gondii DNA was extracted from cell culture-derived tachyzoites and strain typing was performed using the 10 PCR-restriction fragment length polymorphism (RFLP) markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico (Su et al., 2006; Su and Dubey, 2009).

2.7. DNA sequencing of new atypical T. gondii strains

Goat strains representing the five new genotypes were sequenced for four introns (EF1, HP2, UPRT1 and UPRT7) and two genes (GRA6 and GRA7). The target sequences were amplified by PCR. Primers for PCR amplification and sequencing are listed in Supplementary Table S1. PCR products were purified and sequenced from one end using sequencing primers. Sequences were

processed using BioEdit (available free-of-charge at http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and aligned with previously published sequences (Khan et al., 2007, 2011).

3. Results

3.1. Serological prevalence

Antibodies to *T. gondii* were found in 125 (53.4%) of 234 goats, with titers of 1:5 in 20, 1:10 in 44, 1:20 in 16, 1:40 in five, 1:160 in five, 1:320 in five, and 1:640 or higher in 30 goats (Table 1).

3.2. Isolation of T. gondii

Toxoplasma gondii was isolated from 29 of 112 goats; from hearts of one of 46 with titers of <1:5, one of nine with titers of 1:10, one of three with titers of 1:40, and 26 of 40 with titers of 1:160 or higher (Table 1). Four isolates were obtained by bioassays in BALB/c mice or SW mice, six by bioassay in KO mice, and 19 by both KO and SW or BALB/c mice. The percentage of KO mice (68.6%, 35 of 51) that became infected with *T. gondii* was higher than that of the SW or BALB/c mice (51.6%, 48 of 93) (Table 2).

3.3. Virulence of T. gondii isolates

Three of the 29 isolates (TgGoatUs 4, 6, 26; Table 3) were mildly virulent to SW or BALB/c mice. Details of isolation from these isolates are as follows.

3.3.1. TgGoatUs 4

Tissue cysts were found in one of the three BALB/c mice killed 60 days after they had been inoculated with heart of goat 29. Brain homogenate of the infected mouse was inoculated s.c. into four SW mice and one KO mouse; these mice died (or were killed when comatose) of acute toxoplasmosis 11–13 days p.i. and their tissues were fed to cat 70 to obtain oocysts. Mortality data after inoculation with oocysts and tachyzoites are shown in Table 3.

3.3.2. TgGoatUs 6

All three BALB/c mice inoculated with heart homogenate of goat 41 died of toxoplasmosis, 18–27 days p.i. The two KO mice died of toxoplasmosis 16 day p.i. Lung homogenate from one of the KO mouse was inoculated into one SW and one KO mouse. Both mice died day 14 p.i. and their tissues were fed to cat 66 to obtain oocysts. This strain was virulent for mice; all mice inoculated with oocysts died of acute toxoplasmosis, irrespective of the dose (Table 3). All but one mouse inoculated with live tachyzoites survived; this mouse was sick when killed 68 days p.i., and had many tissue cysts in its brain.

3.3.3. TgGoatUs 26

One of the three SW mice inoculated with heart of goat 205 died day 23 p.i. but *T. gondii* was not found microscopically in its brain or lung. Its tissues were inoculated into one SW and one KO mouse; both died of acute toxoplasmosis 10 (KO) and 14 (SW) days p.i. Tissues from the SW mouse that died 14 days p.i. were inoculated into four SW mice; all mice died or were killed 16 days p.i. because they were comatose; tissues of these four mice were fed to cat 76 to obtain oocysts.

All SW mice inoculated with infective *T. gondii* died of acute toxoplasmosis and tachyzoites were found in lungs of all dead mice. Neither *T. gondii* antibodies nor tissue cysts were found in mice that survived. The infectivity of oocysts by the oral and s.c. route was similar and the day of death was correlated with the dose (Table 3). Most of the mice inoculated with tachyzoites died around the third week and the day of death was not correlated with dose.

The groups of SW mice inoculated orally with 1000 oocysts of the isolates TgGoatUS2 (Type II) and TgGoatUS13 (atypical) died between 7 and 13 days p.i.; mice inoculated with lower dilutions (1–100 oocysts) survived and tissue cysts were found in their brains (data not shown).

3.4. Genotyping

Genotyping of the 29 *T. gondii* isolates using the 10 PCR-RFLP markers revealed 12 genotypes. Nine isolates were clonal Type II lineage. Four isolates had type II alleles at all loci except a type I allele at the Apico locus. Four of the 29 isolates were clonal Type III. The remaining 12 strains were divided into nine atypical genotypes; five (TgGoat3, 5, 8, 20, 26) of them are new genotypes (Table 4).

3.5. DNA sequencing

DNA sequences were generated for the five new genotypes at all loci except TgGoatUs3 at the EF1 locus. Comparing these sequences with previously published data (Khan et al., 2007, 2011) revealed identical sequences for all isolates at all loci (Table 5). GenBank database accession numbers for these DNA sequences are shown in Table 5. TgGoatUs3 has combined alleles from Types I, III and the P89 strain. P89 (also known as TgPgUs15) is a pig strain from the USA (Velmurugan et al., 2009). It belongs to a common lineage (Type BrIII) in Brazil (Pena et al., 2008). TgGoatUs5 has combined alleles from Types I and III lineages. TgGoatUs8 has combined alleles from III and the TgCatBr3 strain. Since there is no sequence data for P89 at locus GRA6, we do not know whether TgCatBr3 and P89 are identical at this locus. However, given that P89 and TgCatBr3 are identical at 10 RFLP markers and belong to the Type BrIII lineage in Brazil, it is likely that both will have an identical allele at the GRA6 locus. TgGoatUs20 has combined alleles from Types I, II and the COUGAR (also known as TgCgCa1) strains. TgGoatUs26 has combined alleles from Types I and III lineages.

4. Discussion

In the present study, seroprevalence of *T. gondii* was high, even at a titer of 1:40. We screened heart fluid starting at a low (1:5) dilution, because the MAT titer that should be considered specific for the detection of antibodies to *T. gondii* in goats has not been determined. In the present study, results were based on blood clot/fluid from the heart that had been stored for 1–4 days after slaughter of goats. The MAT pattern was not normal at low dilutions (<1:40). Therefore, we are uncertain concerning the specificity of these low level antibodies. Richomme et al. (2009), using the same serological procedure as used in the present study, isolated viable *T. gondii* from 11 (45.8%) of 24 pigs with a titer of 1:6 and 1:12; among these six were bioassay-positive at 1:6 (Richomme et al., 2009; Villena and Gilot-Fromont, personal communication).

In the present study, *T. gondii* was isolated from hearts of two of 69 (2.8%) goats with titers of <1:40, and 27 of 53 (50.9%) goats with titers of 1:40 or higher (Table 1). Ragozo et al. (2009) isolated viable *T. gondii* from tissues of two of eight goats with an MAT titer of 1:25. Viable *T. gondii* has been recovered from approximately 3% of seronegative pigs (Dubey et al., 2008a; Dubey, 2009). Although the present study was not designed as a validation study, the results support the validity of MAT (titer 1:25 or higher) for the detection of *T. gondii* in goats.

The mouse bioassay results in the present study suggest that the density of *T. gondii* in myocardium is low because only 57.6% (83 of 144) of mice inoculated with hearts of 29 infected goats

Table 4
Summary of genotyping of *Toxoplasma gondii* isolates from goats from a local retail meat store in Maryland, USA.

Isolate designation	Genotype (ToxoDB Genotype #)[g]	PCR-RFLP markers										
		SAG1	5′ + 3′ SAG2[e]	SAG2[f]	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
RH,GT1	Type I (ToxoDB #10)	I	I	I	I	I	I	I	I	I	I	I
PTG	Type II (ToxoDB #1)	II or III[a]	II	II	II	II	II	II	II	II	II	II
CTG	Type III (ToxoDB #2)	II or III	III	III	III	III	III	III	III	III	III	III
TgCgCa1 (COUGAR)	Reference (ToxoDB #66)	I	II	II	III	II	II	II	u-1	I	u-2	I
MAS	Reference (ToxoDB #17)	u-1	I	II	III	III	III	u-1	I	I	III	I
TgCatBr5	Reference (ToxoDB #19)	I	III	III	III	III	III	I	I	I	u-1	I
TgCatBr64	Reference (ToxoDB #111)	I	I	u-1	III	III	III	u-1	I	III	III	I
TgRsCr1	Reference (ToxoDB #52)	u-1	I	II	III	I	III	u-2	I	I	III	I
TgGoatUS2, 9,22,27	Type II (ToxoDB #3) ($n = 4$)	II or III	II	II	II	II	II	II	II	II	II	I
TgGoatUS1,11,12,14,15,16,18,19,21	Type II, Clonal (ToxoDB #1) ($n = 9$)	II or III	II	II	II	II	II	II	II	II	II	II
TgGoatUS4,10,23,29	Type III, Clonal (ToxoDB #2) ($n = 4$)	II or III	III	III	III	III	III	III	III	III	III	III
TgGoatUS3	Atypical #1 (ToxoDB #156)	I	III	III	III	I	I	III	III	III	I	III
TgGoatUS5	Atypical #2 (ToxoDB #143)	I	I	I	III	I	III	III	I	III	I	III
TgGoatUS8	Atypical #3 (ToxoDB #170)	II or III	III	III	III	I	III	III	III	III	III	III
TgGoatUS7	Atypical #4[a] (ToxoDB #39)	II or III	II	II	II	II	II	II	II	I	II	II
TgGoatUS13,17,24	Atypical #5[b] (ToxoDB #4) ($n = 3$)	II or III	II	II	II	II	II	II	II	I	II	I
TgGoatUS20	Atypical #6 (ToxoDB #154)	I	II	II	III	II	II	II	u-1	III	II	I
TgGoatUS6	Atypical #7[c] (ToxoDB #118)	I	III	III	III	III	I	I	III	III	I	III
TgGoatUS25,28	Atypical #8[d] (ToxoDB #5) ($n = 2$)	u-1	II	II	II	II	II	II	II	I	II	I
TgGoatUS26	Atypical #9 (ToxoDB #167)	II or III	I	I	I	I	I	I	III	III	III	III

RFLP, restriction fragment length polymorphism.
[a] Previously identified in sheep (Dubey et al., 2008b) and sea otters (Sundar et al., 2008) from the USA.
[b] Previously identified in sheep (Dubey et al., 2008b), white-tailed deer (Dubey et al., 2008c) and pigs (Velmurugan et al., 2009) from the USA, and in dogs from Sri Lanka (Dubey et al., 2007c).
[c] Previously identified in cast from Puerto Rico (Dubey et al., 2007b).
[d] Previously identified in sheep (Dubey et al., 2008b), pigs (Velmurugan et al., 2009) and sea otters (Sundar et al., 2008) from the USA.
[e] Howe et al. (1997).
[f] Su et al. (2006).
[g] ToxoDB Genotype # is available online at ToxoDB.org.

Table 5
Summary of DNA sequence analysis of new atypical *Toxoplasma gondii* strains found in this study.

Toxoplasma gondii isolates	Introns				Genes	
	EF1[a] 511 bp	HP2[b] 493 bp	UPRT1[c] 401 bp	UPRT7[d] 500 bp	GRA6[e] 411 bp	GRA7[f] 420 bp
TgGoatUs3	ND	CTG (III)	P89	P89	RH (I)	CTG (III)
TgGoatUs5	RH (I)	CTG (III)	CTG (III)	CTG (III)	CTG (III)	CTG (III)
TgGoatUs8	CTG (III)	CTG (III)	CTG (III)	CTG (III)	TgCatBr3	CTG (III)
TgGoatUs20	RH (I)	RH (I)	COUGAR	COUGAR	Me49 (II)	COUGAR
TgGoatUs26	RH (I)	CTG (III)	CTG (III)	CTG (III)	RH (I)	RH (I)

DNA sequences from goat strains were compared with previously published data (Khan et al., 2007, 2009) and the identical strains are listed in the table. Roman numerals in parentheses indicate the typical lineages (types I, II and III).
[a] HQ852146, HQ852147, HQ852148, HQ852149.
[b] HQ852160, HQ852161, HQ852162, HQ852163, HQ852164.
[c] HQ852165, HQ852166, HQ852167, HQ852168, HQ852169.
[d] HQ852170, HQ852171, HQ852172, HQ852173, HQ852174.
[e] HQ852150, HQ852151, HQ852152, HQ852153, HQ852154.
[f] HQ852155, HQ852156, HQ852157, HQ852158, HQ852159.

acquired toxoplasmosis (Table 2). In this respect, the percentage of KO mice (68.6%, 35 of 51) that became infected with *T. gondii* was higher than that of the SW or BALB/c (51.6%, 48 of 93) mice (Table 2).

For the present study, heart tissue was selected for bioassay for convenience and availability, since matching serum and tissue from the same animal were not available. In the current study, fluid was removed from the heart, thus minimising chances of error in matching tissue and serum. Goat hearts are edible and are sold commercially.

The 2010 inventory of meat goats in the USA is more than 2.5 million heads. More than 850,000 goats are slaughtered in the USA each year for food and this number is expected to grow as the immigrant population from areas where goat meat is a diet staple continues to increase. Results of the present study and previous surveys indicate the prevalence of *T. gondii* in goats can be high but the role of ingestion of infected goat meat in the epidemiology of toxoplasmosis in humans remains to be determined. In a retrospective study of 131 mothers who had given birth to children infected with *T. gondii*, 50% recalled having eaten uncooked meat (Boyer et al., 2005) but the meat sources were not identified.

Most *T. gondii* isolates from human and animal sources in North America and Europe have been grouped into one of three clonal lineages including Types I, II and III (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002a,b). When tachyzoites were used to infect outbred mice, Type I strains were uniformly lethal. In contrast, Types II and III strains were significantly less virulent (Howe et al., 1996). In the present study, two strains (TgGoatUS6, TgGoatUS26) were highly mouse virulent and both had atypical genotypes with Type I and Type III alleles at several

markers (Table 4). However, 1000 oocysts of the remaining three isolates tested (a Type II, a Type III and an atypical strain) were also lethal to mice, suggesting that all *T. gondii* are potentially pathogenic, depending on the route, dose and the stage inoculated.

Toxoplasma gondii was considered to be clonal with low genetic diversity (Howe and Sibley, 1995). However, we recently found that the isolates of *T. gondii* from Brazil and Colombia are biologically and genetically different from those in North America and Europe (Dubey et al., 2002, 2007a; Lehmann et al., 2006; Dubey and Su, 2009). *Toxoplasma gondii* isolates from asymptomatic chickens from Brazil were in general more pathogenic to mice than isolates from Europe or North America. Additionally, most isolates from chickens in Brazil were different from the major clonal lineages in North America and Europe, and the Type II strain was absent or rare (Dubey and Su, 2009). This study showed the dominance of atypical strains (55%) infecting goats in the USA. Only 31% (9/29) of goats were infected with clonal Type II strains and this is well below the percentage recovered from other domestic animals studied in the USA. The atypical isolates were divided into nine genotypes including five new genotypes (TgGoatUS3, 5, 8, 20, 26 each represent a new genotype) and four previously identified genotypes. Three of the four previously identified genotypes were reported in sheep (Dubey et al., 2008b), sea otters (Sundar et al., 2008), white-tailed deer (Dubey et al., 2008c) or pigs (Velmurugan et al., 2009) from USA or in dogs from Sri Lanka (Dubey et al., 2007c). One of these four previously identified genotypes was reported from Puerto Rico (Dubey et al., 2007b) (Table 3). These data suggest that a few *T. gondii* genotypes are circulating in domestic and wild animals within broad geographical areas.

Little information is available concerning genotypes of *T. gondii* circulating in goats worldwide. Dubey (1980) isolated a mouse virulent strain (GT1) from muscles of a goat from Ohio, USA; the genome of this isolate has been described (Khan et al., 2005). In the present study, there was no Type I strain isolated. Ragozo et al. (2009) isolated viable *T. gondii* from tissues of 12 of 26 seropositive (MAT, 1:25 or higher) goats from Brazil; 10 of these isolates were virulent to outbred mice. These 12 isolates were grouped into five atypical genotypes (four belong to type Br1 and eight belong to unreported types); clonal Types II and III were absent (Ragozo et al., 2010). Thus, the isolates from goats from Brazil were genetically different from isolates from goats in the present study.

Genotyping results from this study showed the dominance of Type II strains in goats in the USA. The second most common type is the Type III lineage. This is in agreement with findings in pigs in the USA (Velmurugan et al., 2009). DNA sequence data confirmed RFLP results in that the newly indentified genotypes in goats have combinations of a few alleles at different loci, suggesting limited diversity at sequence level, and these new genotypes might be derived from only a few ancestral lineages. Results of the present study, and other recent studies, indicate that atypical genotypes of *T. gondii* circulate in the food animal chain in the USA, including lambs (Dubey et al., 2008b), pigs (Dubey et al., 2008a; Velmurugan et al., 2009), and wildlife (Dubey et al., 2007d, 2010), and they are not rare. Genotyping data on *T. gondii* strains from goats worldwide is very limited. Recently, Mercier et al. (2011) isolated *T. gondii* from 10 seropositive (MAT, 1:800) goats from Dienga, Gabon, Africa; all 10 isolates were avirulent for mice and Type III by *T. gondii* microsatellite markers. These differences may be due to different population structures in different geographical regions. Regardless of genotypes identified, all of these studies suggest that goats can be important hosts for *T. gondii* transmission to humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2011.03.006.

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