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Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland

A.E. Berger-Schoch[a], D.C. Herrmann[b], G. Schares[b], N. Müller[a], D. Bernet[c], B. Gottstein[a], C.F. Frey[a,*]

[a] *Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland*
[b] *Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Seestrasse 55, 16868 Wusterhausen, Germany*
[c] *Federal Veterinary Office, Schwarzenburgstrasse 155, 3097 Bern-Liebefeld, Switzerland*

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ABSTRACT

The protozoan parasite *Toxoplasma gondii* infects almost all warm blooded animal species including humans, and is one of the most prevalent zoonotic parasites worldwide. Postnatal infection in humans is acquired through oral uptake of sporulated *T. gondii* oocysts or by ingestion of parasite tissue cysts upon consumption of raw or undercooked meat. This study was undertaken to determine the prevalence of oocyst-shedding by cats and to assess the level of infection with *T. gondii* in meat-producing animals in Switzerland via detection of genomic DNA (gDNA) in muscle samples. In total, 252 cats (44 stray cats, 171 pet cats, 37 cats with gastrointestinal disorders) were analysed coproscopically, and subsequently species-specific identification of *T. gondii* oocysts was achieved by Polymerase Chain Reaction (PCR). Furthermore, diaphragm samples of 270 domestic pigs (120 adults, 50 finishing, and 100 free-range animals), 150 wild boar, 250 sheep (150 adults and 100 lambs) and 406 cattle (47 calves, 129 heifers, 100 bulls, and 130 adult cows) were investigated by *T. gondii*-specific real-time PCR. For the first time in Switzerland, PCR-positive samples were subsequently genotyped using nine PCR-restriction fragment length polymorphism (PCR-RFLP) loci (SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico) for analysis. Only one of the cats shed *T. gondii* oocysts, corresponding to a *T. gondii* prevalence of 0.4% (95% CI: 0.0–2.2%). In meat-producing animals, gDNA prevalence was lowest in wild boar (0.7%; 95% CI: 0.0–3.7%), followed by sheep (2.0%; 95% CI: 0.1–4.6%) and pigs (2.2%; 95% CI: 0.8–4.8%). The highest prevalence was found in cattle (4.7%; 95% CI: 2.8–7.2%), mainly due to the high prevalence of 29.8% in young calves. With regard to housing conditions, conventional fattening pigs and free-range pigs surprisingly exhibited the same prevalence (2.0%; 95% CI: 0.2–7.0%). Genotyping of oocysts shed by the cat showed *T. gondii* with clonal Type II alleles and the Apico I allele. *T. gondii* with clonal Type II alleles were also predominantly observed in sheep, while *T. gondii* with mixed or atypical allele combinations were very rare in sheep. In pigs and cattle however, genotyping of *T. gondii* was often incomplete. These findings suggested that cattle in Switzerland might be infected with *Toxoplasma* of the clonal Types I or III, atypical *T. gondii* or more than one clonal Type.

1. Introduction

Toxoplasma gondii is one of the most prevalent zoonotic parasites worldwide. While only felidae can act as definitive hosts and thus shed oocysts in their faeces, almost all

* Corresponding authors.
Tel.: +41 31 631 2475; fax: +41 31 631 2477/2622.
E-mail address: caroline.frey@ipa.unibe.ch (C.F. Frey).

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warm-blooded animals can serve as intermediate hosts. These, upon primary infection, will first undergo a tachyzoite stage infection by the parasite, followed by the formation of bradyzoite-containing tissue cysts, primarily in brain or muscles (Dubey and Beattie, 1988; Dubey, 1996; reviewed by Tenter et al., 2000). Humans may acquire a *T. gondii* infection via (1) oral uptake of sporulated oocysts from the environment, (2) consumption of raw or undercooked meat containing tissue cysts or (3) via transplacental transmission of the parasite from the non-immune mother to the foetus. Studies in Europe have shown that 35–58% of women at child-bearing age were seropositive for *T. gondii* (reviewed by Tenter et al., 2000). In Switzerland, 46% of women at child-bearing age presented a seropositive immune status (Jacquier et al., 1995), but seroprevalences are likely to have decreased in the last decade, following the general trend in Europe (reviewed in Pappas et al., 2009). In fact, two locally restricted studies carried out with women in child-bearing age in Switzerland detected seroprevalences of 35% and 8.2%, respectively (Signorell et al., 2006; Zufferey et al., 2007). In Europe, congenital toxoplasmosis affected approximately 1–10 out of 10,000 newborns, of whom 1–2% suffered from general health problems or even died and 4–27% developed ocular disease (Cook et al., 2000). The European Food Safety Authority (EFSA) has recognized toxoplasmosis as the parasitic zoonosis with the highest human incidence and has recently published a scientific opinion that clearly states the need of representative data on the occurrence of toxoplasmosis and the distribution of the parasite in Europe (EFSA, 2007). Recently, different options of obtaining *T. gondii*-free meat have been discussed (Kijlstra and Jongert, 2008). It is thus of great importance to collect reliable data on the actual prevalence of *T. gondii* in meat-producing animals, but also on the prevalence of oocyst-shedding by cats to assess the infection-risk for the human population.

In North America and Europe, mainly three *T. gondii* clonal lineages dominate, designated as clonal Types I, II and III based on PCR-restriction fragment length polymorphism (PCR-RFLP) and microsatellite typing (Howe and Sibley, 1995; Ajzenberg et al., 2002). These clonal types exhibit different levels of virulence in outbred mice. While clonal Type I always causes a lethal infection, the other two clonal types are far less virulent (Sibley and Boothroyd, 1992). In South America and Asia, however, *T. gondii* genotypes are predominantly atypical and of mixed clonal Types (Lehmann et al., 2006; Dubey et al., 2007). Such genotypes could arise when a feline host ingests prey infected with *T. gondii* of more than one of the three clonal Types I, II, or III. In these cases, the clonal types may undergo sexual recombination in the feline gut and the resulting progeny would thus represent a mixture of the two parental genotypes (Su et al., 2002; Saeij et al., 2006). More recently, *T. gondii* with atypical allele combinations have also been observed in Germany (Herrmann et al., 2010). As yet, nothing is known about the *T. gondii* genotypes present in the animal populations in Switzerland.

The aim of this study was to assess the prevalence of *T. gondii* oocyst-shedding in cats, using faecal samples obtained from animal shelters, catteries and routine diagnostic samples at the Institute of Parasitology in Bern, as well as the prevalence of *T. gondii* gDNA in diaphragm samples of cattle, sheep and pigs. In addition, and for the first time in Switzerland, genotyping of oocysts shed by cats and parasite gDNA detected in muscles of meat-producing animals was carried out. Furthermore, parasite gDNA detected in muscle of meat producing animals of a previous study (Wyss et al., 2000) was subjected to genotyping because at the time of that former study, genotyping was not yet done.

2. Materials and methods

2.1. Diagnostic samples

A total of 252 cat faecal samples were collected between January 2007 and August 2008. Animal shelters and catteries in the region of Bern and Olten were contacted and offered a free coproscopical examination for their cats. Furthermore, cat faeces entering the routine analysis of the Institute of Parasitology in Bern were included in the study. Thus, faeces were obtained from stray animals ($n = 43$, 17%), healthy pet cats ($n = 171$, 68%) and of cats with diarrhoea or other gastrointestinal disorders ($n = 38$, 15%). 66 cats (26%) were young cats aged up to 2 years, 124 cats (49%) were adult cats (>2–10 years) and 52 cats (21%) were old cats aged over 10 years. Of 10 cats (4%) information on age was missing.

Diaphragm samples of three animal species were collected at slaughterhouses: the two most frequently consumed meat-producing animals of Swiss residents, pigs and cattle; furthermore sheep, an animal species described to exhibit a conventionally high prevalence for *T. gondii* infection (reviewed by Tenter et al., 2000; Dubey and Jones, 2008; Kijlstra and Jongert, 2008). Wild boar were selected as an out-group control reflecting the environmental contamination in non-urban surroundings. We classified all production animals into different age groups and different housing conditions, where applicable: cows (>2 years old), bulls (>11 months old), heifers (8 months to 2 years old), calves (5–7 months old), ewes (>11 months old), lambs (<11 months old), adult pigs (3–4 years old), finishing pigs (6 months old), free-range pigs (6 months old) and wild boar (all ages). Animals were selected such as to cover the largest possible geographical range in Switzerland. The study did not include any commercial meat imported into Switzerland.

2.2. Sample size for examinations in meat-producing animals

Sample sizes needed to obtain accurate frequencies of *Toxoplasma*-infection were calculated using the freely available software WinEpiscope 2.0 (http://www.clive.ed.ac.uk/clive) with the following values: target population: >50,000 meat-producing animals and wild boar, and 500,000 cats; confidence level: 95%; acceptable error: 8% for the frequency calculation of *T. gondii*-infections in meat-producing animals and wild boar and 1.4% for the calculated frequency of *T. gondii* oocysts in cat faeces; sensitivity and specificity of the diagnostic test were set to 100% for calculation of the sample size due to ignorance of the true values. Because meat-producing animals and

wild boar were sampled both for the presence of antibodies against *T. gondii* in meat juice (Berger-Schoch et al., in press), and the presence of *T. gondii* gDNA (this study), we calculated the necessary sample size based on already available seroprevalence data but also on published data on the molecular detection of *T. gondii* gDNA of the particular animal categories. Data were taken from a previous Swiss study carried out 10 years ago (Wyss et al., 2000), or relied for lambs, free-range pigs and wild boar upon findings in the literature from neighbouring European countries (reviewed by Tenter et al., 2000; Gauss et al., 2005; van der Giessen et al., 2007). As the sample sizes necessary to accurately assess seroprevalences exceeded those needed to assess the frequency of *T. gondii* gDNA, we calculated our sample size based on serological data. The estimated prevalence of cats shedding oocysts were set to 1%, based on the average yearly number of positive coproscopical samples found during the routine diagnostic examinations of the Institute of Parasitology, Bern. Calculated sample sizes were as follows: 200 cats, 120 adult pigs, 24 finishing pigs, 100 free-range pigs, 150 wild boar; 130 cows, 130 heifers, 100 bulls, 6 calves; 150 ewes, 100 lambs. Arbitrarily, for finishing pigs and calves we increased the sample size to 50.

2.3. Sample collection

Freshly shed cat faeces were collected by the persons taking care of the animals and by veterinarians and immediately sent to the Institute of Parasitology in Bern, where they were stored at 4 °C for a maximum of 4 days until further processing.

Diaphragm tissue samples (approximately 22 g in weight) were collected between April 2006 and December 2008 from adult pigs, finishing pigs, free-range pigs, calves, heifers, bulls, cows, lambs and sheep at the five largest Swiss slaughterhouses; these cover the main area of Swiss animal husbandry production. Additionally, samples of free-range pigs and sheep were collected from small slaughterhouses. Up to two animals per original farm were allowed. All samples were immediately refrigerated and subsequently kept at 4 °C. They were then transported once a week to the diagnostic laboratory of the Institute of Parasitology, University of Bern. For analysis, one gram of each sample was carefully dissected, minced into minute pieces, and stored in a sterile vial at −20 °C until further processing. For each sample, a new disposable scalpel blade and forceps were used to avoid cross-over contamination of DNA. Any remaining material was stored separately at −20 °C as a backup. At the slaughterhouse, baseline data were collected on the origin of the animals and, if applicable, on the production label under which they had been produced. Diaphragm tissue samples originating from wild boar were directly sent to the Institute of Parasitology in Bern by hunters as part of the official meat inspection scheme for trichinellosis. Thus, the spatial distribution and age of these animals was arbitrary and not planned in advance as part of the study design. On the other hand, we assumed that the wild boar sample collection was representative of the hunted and consumed meat from wild boar.

2.4. Coproscopical methods

All faecal samples of cats were analysed using a combined sedimentation/flotation method with 44% zinc chloride solution (w/v) with a specific gravity of 1.3 (Bauer, 2006). The processed diagnostic material was transferred to microscopic glass slides and examined by light microscopy using a magnification of at least 200×. When oocysts of a diameter of 9–14 μm were detected, the remaining sample was processed by a flotation method using a saturated sucrose solution with a specific gravity of 1.3 instead of zinc chloride in order to avoid possible adverse effects of the zinc chloride in the following DNA purification and PCR steps (Schares et al., 2005). gDNA was subsequently isolated from purified oocysts with the DNeasy Kit according to the manufacturer's instructions (Qiagen, Switzerland).

2.5. gDNA extraction of meat

gDNA of minced meat samples was isolated using the DNeasy Kit (Qiagen, Switzerland) with the following modification in order to extract gDNA from 2 × 500 μg tissue per sample: per 500 μg tissue 100 μl proteinase K and 900 μl ATL buffer was added, followed by an over-night incubation at 56 °C. This protocol ensured that after digestion no visible meat-fibres were observed. 100 μl of the digested material was then further processed according to the manufacturer's instructions.

2.6. PCR and genotyping of T. gondii

The procedure for *T. gondii*-specific real-time PCR was carried out as described earlier using forward primer 5′-GGAGGACTGGCAACCTGGTGTCG-3′ and reverse primer 5′-TTGTTTCACCCGGACCGTTTAGCAG-3′ (Costa et al., 2000; Scheidegger et al., 2005). The analytical sensitivity of the PCR allowed detection of less than one oocyst, respectively of one tachyzoite/bradyzoite as assessed by amplification reactions with gDNA-preparations of serial dilutions of *T. gondii* (Costa et al., 2000). As a positive control for the PCR, gDNA equivalents to approximately 10 parasites were used.

Oocysts of a diameter of 9–14 μm of faecal origin were additionally analysed by a *Hammondia hammondi*-specific PCR as previously described (Schares et al., 2008).

All *T. gondii*-positive samples were submitted to genotyping. Furthermore, 13 gDNA samples originating from an earlier study (Wyss et al., 2000) were also included; these had been derived from meat of sheep (six samples), cows (two samples), heifers (three samples), calves (one sample) and bulls (one sample). Genotyping was performed by PCR-RFLP using genetic markers for SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico as described previously (Su et al., 2006; Dubey et al., 2007; Herrmann et al., 2010). Restriction of PCR-amplified marker regions was performed as described by Su et al. (2006) with restriction enzymes purchased from Fermentas, Germany. Each PCR-RFLP analysis included a positive and negative control alongside samples to be analysed.

Table 1
Prevalence of infection with *Toxoplasma gondii* as assessed by real-time PCR with point estimate (*f*) and 95% confidence limits of the binomial distribution (95% CI) for cats (oocysts), pigs, wild boar, cattle and sheep (meat) as assessed by real-time PCR.

Category	Sample size	PCR-positive animals		
		n	*f* (%)	95% CI
Cats	252	1	0.4	0.0–2.2
Pigs (all groups)	270	6	2.2	0.8–4.8
Finishing pigs	50	1	2.0	0.2–7.0
Mother sows	120	3	2.5	0.5–7.1
Free-range pigs	100	2	2.0	0.2–7.0
Wild boar	150	1	0.7	0.0–3.7
Cattle (all groups)	406	19	4.7	2.8–7.2
Calves	47	14	29.8*	17.3–44.9*
Heifers	129	0	0.0	0.0–2.8
Bulls	100	0	0.0	0.0–2.8
Cows	130	5	3.8	1.3–8.7
Sheep (all groups)	250	5	2.0	0.1–4.6
Lambs	100	0	0.0	0.0–3.6
Ewes	150	5	3.3	1.1–7.6

* Statistically significantly higher prevalence than heifers, bulls and cows ($P < 0.00001$).

2.7. Statistical analyses

Statistical analyses were performed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and NCSS 2007 (NCSS Statistical Software, Kaysville, Utah, USA). Prevalences with 95% confidence limits of the binomial distribution were derived by species and (within those) for the different age groups and production types as applicable. Prevalence differences between groups were assessed using the two-tailed Fisher's exact test with Bonferroni correction.

3. Results

Cats: Out of 252 faecal samples analysed, two samples contained oocysts of a diameter of 9–14 μm. PCR analysis of the recovered oocysts revealed one sample to be *T. gondii*, while the other sample was identified as *H. hammondi*. Thus, the prevalence of cats shedding *T. gondii* oocysts was 0.4% (95% CI: 0.0–2.2%) (Table 1). The cat shedding *T. gondii* oocysts was 11 years old and suffered from pneumonia accompanied by vomiting and anorexia. It was a pet cat with no outdoor access.

Porcines: Three of 120 mother sows (2.5%; 95% CI: 0.5–7.1%), one of 50 finishing pigs (2.0%; 95% CI: 0.2–7.0%), two of 100 free-range pigs (2.0%; 95% CI: 0.2–7.0%) and one of 150 wild boar (0.7%; 95% CI: 0.0–3.7%) were PCR-positive for *T. gondii* gDNA (Table 1). No significant differences were observed between the different groups ($P > 0.33$).

Bovines: Five of 130 cows (3.8%; 95% CI: 1.3–8.7%), none of 100 bulls and 129 heifers (0.0%; 95% CI: 0.0–2.8%), and 14 of 47 calves (29.8%; 95% CI: 17.3–44.9%) were PCR-positive for *T. gondii* gDNA (Table 1). Calves had a significantly higher prevalence than the other groups ($P < 0.00001$).

Ovines: Five of 150 adult sheep (3.3%; 95% CI: 1.1–7.6%) and none of 100 lambs (0%; 95% CI: 0.0–3.6%) were PCR-positive for *T. gondii* gDNA (Table 1). The difference between the two groups was not statistically significant ($P = 0.16$).

Geographically, the positive animals of all assessed species were evenly dispersed all over the sampled areas (data not shown). Thus, there were no regions with an evident accumulation of positive animals.

Genotyping: The *T. gondii* oocysts isolated from the cat had clonal Type II alleles at all loci except a clonal Type I allele at the locus Apico (Table 2). All gDNAs of positive meat samples and 13 positive samples originating from a prior study (Wyss et al., 2000) were submitted to genotyping but this was only successful in a limited number of specimen. The analyses of five sheep samples demonstrated that two animals were infected with *T. gondii* clonal Type II, Apico I. A third sheep showed alleles of clonal Type II at five loci (SAG2, SAG3, c22-8, L358 and PK1) and a clonal Type I allele at the locus Apico, whereas three loci could not be PCR-amplified (Table 2). However, 3′SAG2 and 5′SAG2 taken together suggest clonal Type I at locus SAG2, whereas nSAG2 suggests clonal Type II at locus SAG2. Taking all alleles from all marker regions into account this suggests the sheep might be infected with *T. gondii* displaying an atypical allele combination. One sheep (sheep 1410) seemed to be infected with more than one clonal Type or with different recombinant *T. gondii* Types having more than one allele, namely of clonal Type II and III, at locus SAG2. *T. gondii* observed in another sheep (sheep 1419) had either Type II or Type III alleles at different loci, in addition to showing more than one allele (Type I and III) at the locus SAG3 (Table 2).

Table 2
Multilocus genotyping of *Toxoplasma gondii* DNA by PCR-restriction length polymorphism (RFLP) of a cat, a free-range pig and sheep from Switzerland.

Animal ID	PCR-RFLP genotype (genetic marker)										
	nSAG2[a]	3′SAG2	5′SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
Cat	II	II	I or II	II	II	II	II	II	II	II	I
Free-range pig F24	II	II	I or II	*nd*	*nd*	II	*nd*	*nd*	*nd*	*nd*	*nd*
Sheep 1319	II	I or III	I or II	II	*nd*	*nd*	II	*nd*	II	II	I
Sheep 1392	II	II	I or II	II	II	II	II	II	II	II	I
Sheep 1406	II	II	I or II	II	II	II	II	II	II	II	I
Sheep 1410	II + III	II	III	II	II	II	II	II	II	II	I
Sheep 1419	II	*nd*	*nd*	I + III	III	II	*nd*	*nd*	*nd*	*nd*	II

nd marker region could not be amplified by PCR.

[a] nSAG2 marker is based on the 5′-end of the SAG2 gene sequence (Su et al., 2006).

Table 3
Multilocus genotyping of *Toxoplasma gondii* DNA by PCR-restriction length polymorphism (RFLP) of bovines from Switzerland.

Animal ID	PCR-RFLP genotype (genetic marker)										
	nSAG2[a]	3′SAG2	5′SAG2	SAG3	BTUB	GRA6	c22–8	c29–2	L358	PK1	Apico
Calf B4	*nd*	I or III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	I
Calf B24	*nd*	I or III	*nd*	II + III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Calf B37	*nd*	I or III	*nd*	*nd*	*nd*	II	*nd*	*nd*	*nd*	*nd*	I
Calf 512	*nd*	I or III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	I
Heifer 244	II + III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	I
Heifer 246	I	*nd*	*nd*	*nd*	*nd*	III	*nd*	*nd*	*nd*	*nd*	I
Bull 317	*nd*	I or III	*nd*	*nd*	*nd*	I	*nd*	*nd*	*nd*	*nd*	II
Cow K77	*nd*	I or III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Cow 86	*nd*	*nd*	*nd*	III	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*

nd marker region could not be amplified by PCR.
[a] nSAG2 marker is based on the 5′-end of the SAG2 gene sequence (Su et al., 2006).

In pigs, *T. gondii* in only one sample of a free-range pig could be partially genotyped. It resulted in alleles of clonal Type II at two loci (SAG2 and GRA6) but the PCR-amplification of all other loci failed (Table 2).

In *T. gondii* from cattle, only three loci could be amplified by PCR and only in nine samples (Table 3). The other samples contained insufficient amounts of parasite DNA for genotyping. Typing results on *T. gondii*-infected cattle are thus rather inconclusive, but clonal Type I or III alleles were observed in three (calf B4, calf 512, cow K77) and a clonal Type I allele in one (Bull 317) of the bovine samples.

4. Discussion

This study aimed at estimating the prevalence of oocyst-shedding by cats and at assessing the frequency of *T. gondii*-infections in meat-producing animals in Switzerland by detection of gDNA in muscle samples. Diaphragm was chosen because it is easily accessible upon slaughter and has been used in studies in pigs (Gajadhar et al., 1998), sheep (Da Silva and Langoni, 2001) and cattle (Dubey and Streitel, 1976) for the detection of *T. gondii*. We were aware that with using one gram of meat predominantly only animals with high infection intensity would yield positive PCR-findings, thus our data definitively do not reflect a true parasitological prevalence. Nevertheless, a relative, and also comparable, inter-species assessment was possible, as identical amounts of meat were processed for each animal, independently of its species.

The prevalence estimate of oocyst-shedding in cats was 0.4% (95% CI: 0.0–2.2), which was within the range of prevalences reported from neighbouring countries such as Germany (0.11%; 95% CI: 0.1–0.2), Austria (0.1%; 95% CI: 0.0–0.6) and France (0.23%; 95% CI: 0.0–0.8) (Schares et al., 2008). Genotyping of the cat-derived oocysts revealed alleles of clonal Type II with an exception of locus Apico which was clonal Type I. This is in accordance with the results of recent studies from Germany that genotyped *T. gondii* oocysts from various European countries (Schares et al., 2008; Herrmann et al., 2010). A more recent study from Germany identified one isolate as clonal Type III and reported several isolates with atypical allele combinations. The majority of isolates had *T. gondii* clonal Type II alleles except for the locus Apico, which was also often of clonal Type I (Herrmann et al., 2010). Interestingly, the only cat that shed *T. gondii* oocysts in our study was over the age of 10 having no outdoor access. However, the cat suffered from pneumonia accompanied by vomiting and anorexia and therefore it is likely that this cat underwent a recrudescence of an earlier *T. gondii* infection. Oocyst shedding in older cats was also reported from Germany (Schares et al., 2008; Herrmann et al., 2010) and therefore older cats should not be disregarded as potential disseminators of *T. gondii* oocysts, especially when they have outdoor access.

Regarding the prevalence of gDNA in meat of sheep and pigs (2.0% and 2.2%, respectively) our results do not differ significantly from the results of a previous study carried out in Switzerland 10 years ago, in which 4.7% of sheep meat samples and 0.0% of pig meat samples were PCR-positive for *T. gondii*. A possible explanation for the finding of PCR-positives in pigs in the present study, and also for the fact that the prevalence in conventional fattening pigs and in free-range pigs in our study is similar (2.0%), might lie in the implementation of new Swiss animal welfare regulations and corresponding requirements regarding animal-friendly housing systems (Anonymous, 2008). According to these regulations, all pigs must have access to straw or other organic material high in fibres. This system has gradually replaced the old conventional, hyper-hygienic closed intensive maintenance units.

In the European context, a comparable study in England assessing meat by PCR from retail stores found much higher prevalences in both pork and lamb meat, namely 33.3% and 66.6%, respectively (Aspinall et al., 2002). A recent study from France isolated viable *T. gondii* parasites from 42% of adult sheep carcasses and from 2% of lamb carcases (Halos et al., 2009), demonstrating the strong age-effect in the epidemiology of the infection. In accordance with this, we also observed that ewes had a higher prevalence (3.3%) compared to lambs (0.0%), even though the difference was not significant. The much higher prevalences found in the French study can be explained by the fact that the whole heart of the animals was subjected to bioassay, the results are therefore not immediately comparable to our investigations. The study from England, however, extracted DNA from one gram of meat for assessment of *T. gondii* infec-

tion, similar to our study, and the results are thus directly comparable.

Regarding bovines, the prevalence in calves was surprisingly high (29.8%). Whether beef from these PCR-positive animals is infectious to humans remains to be studied. In three of the positive calf samples, *T. gondii* DNA was amplified for genotyping thus giving confidence in the diagnostic real-time PCR results. A possible explanation for the high prevalence might be that the diagnostic PCR may have detected tachyzoite DNA, i.e. these calves may have been sampled at a very early stage after primary infection. Opsteegh et al. (in press) found PCR-positive cattle that were seronegative and concluded from this finding that possibly only recently infected animals obtain a parasite load that is high enough to be detected by direct methods. If the infection would persist in calves, we would expect to find comparable prevalences also in older animals. However, prevalences in heifers, bulls and cows were significantly lower (0.0–3.8%) and were comparable with the prevalences found in Switzerland 10 years ago (Wyss et al., 2000). This suggests that our results regarding the high prevalence in calves should be considered as preliminary results. Viable parasites have not been isolated from beef to date (Dubey et al., 1986, 2005), however various studies have shown that gDNA can be detected in beef by PCR (Wyss et al., 2000; Aspinall et al., 2002; Canada et al., 2002; Moré et al., 2008). The exact fate of *T. gondii* infecting cattle remains therefore uncertain and further research is needed to strengthen our knowledge on *T. gondii* infection in cattle.

Genotyping of an oocyst sample from a cat and two sheep- and one pig-meat samples revealed predominantly alleles of *T. gondii* clonal Type II. For a methodically reliable genotyping, a reasonable amount of parasite DNA is required, which was not always given in all samples of this study (DCH, personal observation). Especially in the bovine and pig samples, where only one or two loci could be PCR amplified, the methodical approach reached its limitations. Therefore limited genotyping results of bovine and pig samples can only provide an indication in terms of genotypes. Some samples revealed more than one allele at a given locus, indicating an infection with more than one, genetically different, genotype of *T. gondii*. Other samples revealed different alleles at a number of loci, indicating infection with atypical *T. gondii*. Results obtained for sheep in Switzerland mainly corresponded with results from other European countries. Studies of *T. gondii* in sheep from the UK (Owen and Trees, 1999), France (Dumetre et al., 2006; Halos et al., 2009) and Denmark (Jungersen et al., 2002) found *T. gondii* of clonal Type II. However, in some studies only a single genetic locus was used for genotyping. It would therefore be possible that isolates previously classified as clonal Type II might well be of an atypical genotype, provided more genetic marker regions would be investigated.

There is also evidence that the population structure of *T. gondii* in Europe and North America is more diverse than previously thought, and that sexual recombination plays an important role in this diversification of *T. gondii* in nature (Grigg and Suzuki, 2003; Ajzenberg et al., 2004; Dubey and Jones, 2008; Dubey et al., 2008b,c), especially in South America (Pena et al., 2008) and Asia (Dubey et al., 2007, 2008a).

Our results also found, apart from clonal Type II *T. gondii* infections, indications for mixed infections or infections with atypical *T. gondii* in sheep of Switzerland.

Infections with more than one genotype can be induced experimentally in cats (Araujo et al., 1997; Dao et al., 2001) but were also shown to occur in naturally infected cats (Herrmann et al., 2010). Grigg and Sundar (2009) suggested that a large diversity of *T. gondii* strains infecting a variety of prey in the sylvatic cycle (e.g. South America) can produce new strains that are capable of clonal expansion and may sweep the domestic cycle. Recombination events could potentially generate more *T. gondii* strains that may possess new biological properties, such as increased virulence for humans (Saeij et al., 2006; Taylor et al., 2006; Grigg, 2007).

5. Conclusions

In conclusion, this study shows that the estimated prevalence of oocyst-shedding in cats from Switzerland is 0.4% (95% CI: 0.0–2.2). gDNA of *T. gondii* has been detected in meat samples of all assessed animals (pigs, ovines and bovines), implicating that the consumption of meat from these species might pose a risk of infection with *T. gondii* in Switzerland. Furthermore, the study allows a first insight into the genotypes of *T. gondii* circulating in Switzerland, revealing that in addition to clonal Type II *T. gondii* there might also be *T. gondii* with mixed and atypical allele combinations present in Switzerland.

Conflict of interest statement

None of the authors of this study has a conflict of interest.

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