ORIGINAL ARTICLE

Toxoplasma gondii in Switzerland: A Serosurvey Based on Meat Juice Analysis of Slaughtered Pigs, Wild Boar, Sheep and Cattle

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Impacts

- *Toxoplasma gondii*, the aetiological agent of toxoplasmosis, is a worldwide occurring protozoan that infects almost all warm blooded animals including man.
- As humans can get infected through the consumption of meat containing parasite tissue cysts, knowledge on the infection prevalence in important food animals is a prerequisite to elaborate prevention strategies.
- Thanks to a serological assay based on meat juice, we could demonstrate that *Toxoplasma gondii* is widespread in Swiss pigs, cattle and sheep and that the prevalence rises with the age of the animals.

Keywords:
Toxoplasma gondii; meat juice; slaughtered animals; P-30-ELISA; Switzerland

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Summary

Toxoplasmosis is one of the most important zoonotic diseases worldwide and is caused by the protozoan *Toxoplasma gondii*. Besides vertical infection during pregnancy, humans can get infected post-natally either by peroral uptake of sporulated *Toxoplasma* oocysts or by ingestion of tissue cysts upon consumption of raw or undercooked meat. The aim of this study was to approximate the risk of human infection via meat consumption by estimating the seroprevalence of *T. gondii* in slaughtered animals in Switzerland and to compare data with prevalences assessed 10 years ago. The study included pigs, cattle, sheep and wild boar of different age groups and housing conditions whenever possible and applicable. A P-30-ELISA was used to detect *T. gondii*-specific antibodies and to determine seroprevalences in meat juice of slaughtered animals. A total of 270 domestic pigs (120 adults, 50 finishing, 100 free-ranging animals), 150 wild boars, 250 sheep (150 adults, 100 lambs) and 406 cattle (47 calves, 129 heifers, 100 bulls, 130 adult cows) were tested. Seropositivity increased with the age of the assessed animals. Independent of the age-group, the overall seroprevalence was lowest in wild boars (6.7%), followed by pigs (23.3%), cattle (45.6%) and sheep (61.6%), respectively. Conventional fattening pigs and free-ranging pigs surprisingly had comparable seroprevalences (14.0% and 13.0%, respectively). Unlike in other European countries, where generally a decrease in the number of seropositive animals had been observed, we found that the prevalence of seropositive animals, when compared with that of 10 years ago, had increased for most species/age groups. Conclusively, the results demonstrated a high seroprevalence of *T. gondii* in animals slaughtered for meat production and revealed that increasing age of the animals is a more important risk factor than housing conditions in Switzerland.

Introduction

Toxoplasmosis is one of the most important zoonotic diseases worldwide and is caused by the protozoan *Toxoplasma gondii*. While only felidae can act as definitive hosts and can thus shed oocysts, almost all warm-blooded animals can serve as intermediate hosts and harbour tissue cysts in brain or muscles (Dubey, 1996; Tenter et al., 2000). Humans can become infected through three routes: (i) uptake of sporulated oocysts from the environment, (ii) consumption of raw or undercooked meat containing tissue cysts and (iii) pre-natal infection. Studies in Europe have shown that 35–58% of women in childbearing age are seropositive for *T. gondii* (Tenter et al., 2000). In Switzerland, 46% of women in that age range were found seropositive (Jacquier et al., 1995). While infection of an immunocompetent person does not generally represent a serious health risk, infection occurring in foetus during pregnancy or in immuno-compromised individuals, can result in severe and potentially life-threatening disease. In Europe, congenital toxoplasmosis affects approximately 1–10 out of 10 000 newborns, of whom 1–2% suffers from general health problems or even die and 4–27% develop an ocular disease (Cook et al., 2000). The European Food Safety Authority (EFSA) has recognized toxoplasmosis as parasitic zoonosis with the highest human incidence and has recently published a scientific opinion that clearly states the need of representative data on toxoplasmosis in Europe (EFSA, 2007). Furthermore, different options of obtaining *Toxoplasma*-free meat are being discussed currently (Kijlstra and Jongert, 2008). Since the most recent study on prevalence of *T. gondii* in slaughtered animals in Switzerland dates back 10 years ago (Wyss et al., 2000), we decided to update our data on this important subject.

The aim of our study was to assess the proportion of animals slaughtered for food production that had circulating antibodies against *T. gondii,* in order to determine the presumptive risk for consumers, acknowledging at the same time that seropositivity does not directly imply infectivity. Conversely, our working hypothesis was based on the assumption that seronegative animals are free of infectious stages of *T. gondii*. We decided to use a serological test for *T. gondii* antibody detection in meat juice, a matrix available upon slaughter that has already been used for the detection of antibodies against *T. gondii* (Wingstrand et al., 1997; Halos et al., 2009) and other zoonotic agents such as *Trichinella* sp. (Nöckler et al., 2005; Frey et al., 2009) and *Salmonella* sp. (Steinbach et al., 2000; Alban et al., 2002). As target species, we selected the two most popular meat sources in Switzerland, namely pork and beef, as well as sheep meat because the ingestion of undercooked lamb is considered an important source of infection for humans (Cook et al., 2000; Dubey, 2009a). Furthermore, these three species have also been assessed before for *T. gondii* antibodies (Wyss et al., 2000) and this assessment offered a convenient opportunity for comparison. In addition to these target groups, free-ranging pigs and wild boars as well as lambs were added to our study population. Different age groups were included in order to assess seroprevalence variations over the host's lifetime and for different housing conditions.

Materials and Methods

Animals

Animal species were selected and categorized in different age groups and different housing conditions, when applicable, as follows: cows (>2 years old), bulls (>11 months old), heifers (8 months–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-ranging pigs (6 months old) and wild boar (all ages).

The criteria for animal selection were adjusted in order to obtain subjects from the largest possible geographical range in Switzerland.

Sample sizes needed were calculated using the freeware software WINEPISCOPE 2.0 (http://www.clive.ed.ac.uk/winepiscope) with the following adjustments: target population: >50 000 animals; confidence level: 95%; acceptable absolute error: ±8%; sensitivity and specificity: 100%. Expected seroprevalences of particular animal categories (cows, bulls, heifers, calves, ewes, adult pigs, finishing pigs) were based on data from a previous Swiss seroprevalence study (Wyss et al., 2000) (Table 1) and for lambs, free-ranging pigs and wild boar on literature from European countries (Tenter et al., 2000; Gauss et al., 2005; van der Giessen et al., 2007), respectively. The statistically calculated sample sizes were as follows: 120 adult pigs, 24 finishing pigs, 100 free-ranging pigs, 150 wild boars; 130 cows, 130 heifers, 100 bulls, 6 calves; 150 ewes, 100 lambs. A minimum of 45 animals per group was arbitrarily determined for finishing pigs and calves.

Sample collection

Diaphragm tissue samples (approximately 22 grams per animal) were collected between April 2006 and December 2008 from adult pigs, finishing pigs, free-ranging pigs, calves, heifers, bulls, cows, lambs and sheep at the five largest Swiss slaughterhouses that receive animals from all regions in Switzerland involved in production animal husbandry. Not more than two animals from the same farm were included. Samples of free-ranging pigs and sheep also were collected at small slaughterhouses. At slaughter, baseline data were collected on the origin of

Table 1. Seroprevalence estimates with exact 95% confidence limits (CI) for *Toxoplasma gondii* antibodies (detected by a P-30-ELISA) in meat juice samples of pigs, cattle and sheep in Switzerland (collected April 2006 – December 2008)

Category	Total	Seropositive animals		Exact 95% confidence limits (CI)	Prevalence (%) in 2000
		N	Prevalence (%)		
Pigs (all groups)	270	63	23.3	18.4–28.8	
Finishing pigs	50	7	14.0*	5.8–26.7	1
Adult pigs	120	43	36.0	27.3–45.1	27
Free-ranging pigs	100	13	13.0	7.1–21.2	NA
Wild boars	150	10	6.7	3.2–11.9	NA
Cattle (all groups)	406	185	45.6	40.6–50.6	
Calves	47	6	12.8	4.8–25.7	4
Heifers	129	48	37.2	28.9–46.2	32
Bulls	100	62	62.0*	51.7–71.5	21
Cows	130	69	53.1*	44.1–61.9	32
Sheep (all groups)	250	154	61.6	55.3–67.7	53
Lambs	100	33	33.0	23.9–43.1	NA
Ewes	150	121	80.7	73.4–86.7	NA

NA = not available.

*Significant increase in seroprevalence (Fisher's exact test, Bonferroni corrected due to multiple testing; $P \leq 0.002$) in comparison to the previous study in 2000.

the animals and, if applicable, on the production label under, which they had been produced. All samples were immediately refrigerated at 4°C and transported once a week to the diagnostic laboratory of the Institute of Parasitology, University of Bern. Diaphragm tissue samples originating from wild boars were directly sent by hunters to the Institute of Parasitology in Bern in the frame of an official meat inspection for trichinellosis. Thus, spatial distribution and age of these animals was arbitrary and not preliminarily designed.

Between 200 and 500 μl of meat juice was obtained by subsequent freezing–thawing of the diaphragm tissue upon arrival at the laboratory as described (Nöckler et al., 2005) and stored at −20°C until tested by a P-30-ELISA.

Serological test

The P-30-ELISA was conducted as described by Wyss et al. (2000), with the exception that meat juice samples were used instead of serum samples. In accordance with the approach described by Wingstrand et al. (1997), who found excellent correlations for *Toxoplasma* antibodies between meat juice and serum in experimentally infected pigs, meat juice samples were 10 times less diluted than serum samples. Briefly, meat juice samples were diluted 1 : 10 and subsequently incubated on 96-well ELISA plates coated with 0.1 μg affinity-purified P-30 antigen of *T. gondii* (SR^2B; Avrillé, France) per well. Positive and negative control sera were obtained from R. Wyss (Bern, Switzerland; cattle), from A. Tenter (Hannover, Germany; sheep) and from P. Lind (Copenhagen, Denmark; porcines). Sera were diluted 1 : 100 and incubated on the same plates as meat juice specimen. Cut-off values were determined separately for each species (cattle, sheep, porcines) according to Wyss et al. (2000) and re-evaluated prior to the testing of our samples with a serum panel of the previous study from 2000. Briefly, the cut-off was set at the mean absorbance value ($A_{405\ nm}$) of 100 negative serum samples plus three standard deviations (SD) (mean A_{405n} + 3 SD). The negative status of the sera used in the validation was previously determined by IFAT and ELISA at the Institute of Parasitology (ITP) in Bern (Wyss et al., 2000). Meat juice samples were attributed arbitrary units (AI) calculated as a function of the positive control and the two negative controls on each plate.

Statistical analyses

Statistical analyses were performed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and NCSS 2007 (NCSS Statistical Software, Kaysville, UT, USA). Seroprevalences with exact 95% confidence limits were obtained for each species and (within those) for the different age groups and production types (whenever relevant). Seroprevalence differences between groups were assessed using the two-tailed Fisher's exact test with Bonferroni correction for multiple comparisons.

Results

Porcines

We found that 36.0% (43 out of 120) of adult pigs, 14.0% (7 out of 50) of finishing pigs, 13.0% (13 out of 100) of free-ranging pigs and 6.7% (10 out of 150) of

wild boars were seropositive for *T. gondii* (Table 1). Adult pigs had a significantly higher seroprevalence than the other groups ($P \leq 0.006$).

The comparison of the results from this study to the sampling campaign in 1999 (Wyss et al., 2000), revealed a significant increase in seroprevalence in finishing pigs from 1% to 14% ($P = 0.002$), but not in adult pigs (27% according to Wyss et al., 2000; $P = 0.24$) (Table 1). Free-ranging pigs and wild boars were not assessed by Wyss et al. (2000) and could therefore not be compared.

Cattle

We found that 53.1% (69 out of 130) of cows, 62.0% (62 out of 100) of bulls, 37.2% (48 out of 129) of heifers and 12.8% (6 out of 47) of calves were seropositive for *T. gondii* (Table 1). Calves had a significantly lower seroprevalence than the other groups ($P < 0.001$) and heifers had a significantly lower seroprevalence than bulls ($P = 0.003$), but after Bonferroni correction showed only a tendency to have a lower seroprevalence compared to cows ($P = 0.013$).

We found significant increases in seroprevalence of bulls from 21% to 62% ($P < 0.00001$) and of cows from 32% to 53.1% ($P = 0.002$) compared to the results of the former study (Wyss et al., 2000) (Table 1).

Sheep

We found that 80.7% (121 out of 150) of adult sheep and 33.0% (33 out of 100) of lambs were positive for *T. gondii* antibodies (Table 1); this difference was highly significant ($P < 0.00001$).

The former study of Wyss et al. (2000) did not specify the age of the assessed sheep. We therefore compared our overall seroprevalence for sheep (61.6%) with the seroprevalence of the former study (53%); this difference was not significant ($P = 0.094$) (Table 1).

The seropositive animals of all assessed species were evenly dispersed all over the sampled areas (Fig. 1) and no areas with an evident accumulation of seropositive animals could be detected.

Discussion

In the present serosurvey for *T. gondii*, the same susceptible meat-producing animals were included to allow a direct comparison between current new findings and data obtained in a study carried out 10 years ago (Wyss et al., 2000). To further enhance comparability, the same test system, a P-30-ELISA, was used again. Different from the study from 10 years ago, we now used meat juice samples, which can be conveniently collected at slaughter and that proved to be suitable for the monitoring of meat-producing animals as demonstrated for *T. gondii* and other parasites (Wingstrand et al., 1997; Lundén et al., 2002; Nöckler et al., 2005; Frey et al., 2009; Halos et al., 2009). Furthermore, the sampling scheme aimed at best representing the consumed meat in Switzerland. Therefore, the estimated seroprevalence obtained can be considered as representative of the true prevalence of *T. gondii* amongst animals consumed in Switzerland.

The overall estimate of seroprevalence of *T. gondii* in sheep, cows and bulls was generally high (>50%). Nevertheless, this count does not necessarily represent a hazard for the population as a seropositive animal does not compulsively harbour active tissue cysts with infective parasites (de A Dos Santos et al., 2005; Halos et al., 2009). The success of isolating live parasites from the meat of seropositive animals may vary considerably: ranging from 95% in experimentally infected pigs (Wingstrand et al., 1997), to 5.4% in naturally infected lambs (Halos et al., 2009), or undetectable in cattle (Dubey et al., 2005). Nevertheless, a seropositive status in herbivore animals is evidence of a contact between the host and *T. gondii* oocysts, except in very young animals that may have obtained maternal antibodies either prenatally or colostrally and thus certainly represents a reliable indicator for the environmental load of *T. gondii* oocysts.

In accordance with other studies, we found an increase in seroprevalence directly proportional to the age of the animals assessed (Damriyasa et al., 2004; Dubey, 2009a,b). Unlike other authors (van der Giessen et al., 2007; Gebreyes et al., 2008) and the previous study of Wyss et al. (2000), we could not find a difference for seroprevalence of *T. gondii* between conventional fattening pigs and free-ranging pigs. In addition, the comparison of the present study with data from the sampling campaign 10 years ago (Wyss et al., 2000) showed a general increase in seroprevalence for the assessed animal species, which was significant in bulls, cows and finishing pigs. This finding is contradictory to trends observed in other countries, where generally, especially in pigs, decreasing prevalences are observed (Dubey, 2009b). A possible explanation lies in the implementation of new Swiss animal welfare regulations and their requirements with regard to animal friendly housing systems (Anonymous, 2008). For instance, 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. For cattle, the allowance of a minimum of 90 days on pasture each year is a new regulation as well. Increased outdoor and pasture access may explain – at least partially – the increased seroprevalence in some meat-producing animals in Switzerland in the last decade.

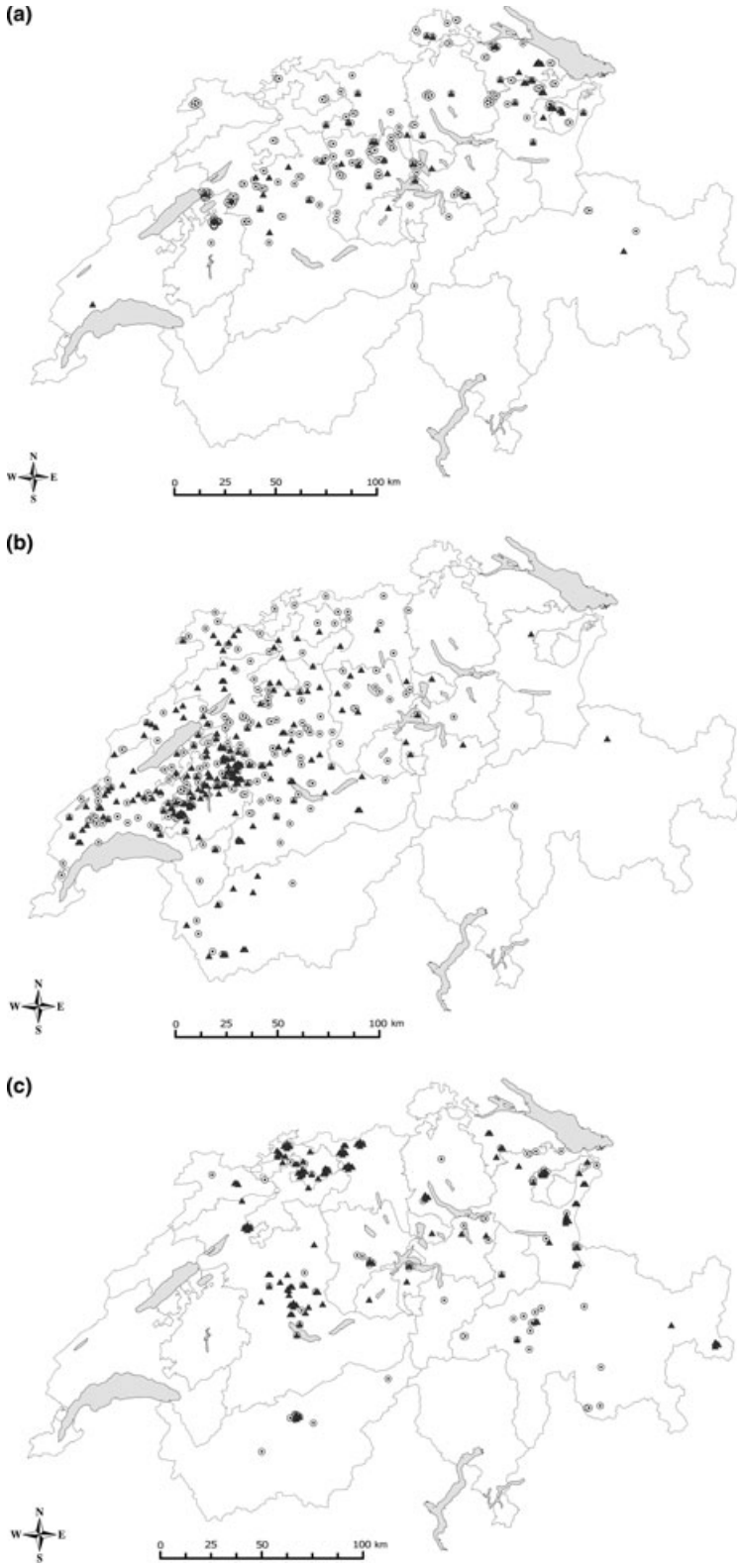

Fig. 1. Geographical distribution of tested animals. (a) Domestic pigs; (b) bovines; (c) sheep. Seropositive animals are indicated as triangles, seronegative animals as circles.

The statistically significant increase in seroprevalences of cows, bulls and fattening pigs might also reflect an increased contamination of the environment (mainly food production or storage places, pastures) with oocysts upon faecal contamination by cats. Cats are the Swiss favourite pet and the Swiss cat population is estimated to approximately 1.35 million animals. Although the largest part of the feline population is restricted to (peri)-urban areas, most of these cats and especially the rural farm cats, have a permanent out door access. The broad geographical distribution of seropositive farm animals and the relatively low seroprevalence in wild boars that usually live in non-populated areas (with a low cat population density) would sustain the hypothesis that the shedding of oocysts by cats is an important factor in *Toxoplasma* epidemiology in Switzerland.

Overall, the results of our study show that *Toxoplasma* infections are prevalent in all assessed animals irrespective of the housing systems. Seroprevalence increases with the age of the slaughtered animals. Pregnant women who are detected seronegative for *T. gondii* and other susceptible categories of people in Switzerland should therefore be very careful when preparing and consuming meat and they should make sure that they strictly apply to the recommendations of food hygiene and safety such as washing hands after the preparation of meat and of eating only well-cooked meat.

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