Chapter 2

Prevalence, Genetic Diversity, Tissue Distribution, and Risk Factors Contributing to *T. gondii* Burden in Domestic Pigs

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Additional information is available at the end of the chapter

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Abstract

Toxoplasma gondii is an obligatory intracellular parasite of mammals, including humans and domestic animals. The infection with this parasite has severe clinical consequences, as it causes abortion or fetal abnormalities, encephalitis in immunocompromised humans, ocular toxoplasmosis with chorioretinitis, and it may contribute to Alzheimer disease. Therefore, an efficient control of *T. gondii* by prevention of the transmission to humans is strongly recommended. Pork is considered as an important source of toxoplasmosis, due to the frequent consumption of the raw or undercooked porcine meat products, a high susceptibility of pigs to the infection, and because of the numerous risk factors, contributing to the prevalence of toxoplasmosis in the pig population. The cellular and humoral immune responses, such as IgM, IgG, IFN-gamma, and interleukin-10 or -12 production, associated with the acute and chronic infection in pigs, do not prevent development of the tissue cysts, which persist lifelong within the intermediate host. Therefore, the prevalence of *T. gondii* in the pig population might be an useful indication of the risk associated with the consumption of the porcine meat.

Keywords: *Toxoplasma gondii*, pigs, meat, risk factors, parasitic burden

1. Introduction

Toxoplasma gondii is an obligate and worldwide-distributed intracellular parasite, causing disease in all warm-blooded animals, which includes aquatic and terrestrial mammals and birds. The prevalence of toxoplasmosis in humans reaches, depending on the study, 33–50% of the worldwide population, regardless of the geographical location or the economic status of the inhabitants [1, 2]. The pig is, among other domestic and wild mammals, a common

intermediate host for *T. gondii*. At the same time, it can trigger human infection via the consumption of raw, undercooked, or cured porcine meat products [3]. Therefore, pork is indicated as one of the major meat sources associated with human food borne *T. gondii* infection, together with *Salmonella* and *Campylobacter* [4].

Although the exact prevalence of this food pathogen, in consumption of meat, is difficult to establish, the subsequent infection rate of humans has been estimated as an average of 300 consumers per 1 infected animal [5]. In addition, nearly all tissues from a pig are used directly for the consumption or processed in other meat products without freezing or cooking, increasing in that way the chance of the transmission of the disease [1].

The naturally infected domestic pigs are considered one of the sources for toxoplasmosis in humans. The lack of an obligatory screening method for the detection of antibodies or the viable parasite in the edible tissues, respectively on farm level or in slaughterhouses, increases the risk that infected animals enter the food chain. The worldwide prevalence of 0.4–93% varies significantly between continents and countries, being determined by diverse serological techniques using different cutoff values. Overall, the results strongly depend on the detection method, the age, and the size of the sampled population. In the developed countries, a spectacular drop in the prevalence of toxoplasmosis was observed in pigs, raised indoor in a strict confinement. Interestingly, next to the management system, many other factors can contribute to the increased risk of infection in pigs in the modern farms. The rodent control is of pivotal importance, together with the proper carcass disposal. The increasing age of the animals, small size of the herd, free range or backyard pigs rather than the strict confinement housing, source of water, and feeding of goat whey to the pigs are frequently listed risk factors. Reversely, organic or biofarms increase the chance of the transmission to pigs, and indirectly, toward humans.

2. Prevalence of *T. gondii* in domestic pig

The global incidence of the porcine toxoplasmosis per continent and country is shown in **Table 1**, taking into account the origin and the age of the animals, the farm management, and the serologic assay applied [48, 49]. According to the report from European Food Safety Agency published in 2012 [50], describing the number of the foodborne zoonotic outbreaks between 2008 and 2010, the highest proportion of positive samples in PCR or serology for *T. gondii* across all countries was reported for sheep and goats. However, the clinical manifestation of toxoplasmosis is particularly obvious in these two species due to abortion, so it is also more likely to be confirmed, compared with other animal species, in which the more subtle signs of infection (particularly in the acute phase of the disease) may be missed. Referring to the same report, the prevalence of *T. gondii*-specific antibodies in the porcine serum samples collected in all the EU-states was 2.2% [50].

In general, the seroprevalence of *T. gondii* dropped significantly during the last decades in Europe and in the USA. Corresponding to that, a low (0.0–0.4%) to moderate (36%) prevalence was estimated on conventional pig farms in European countries [43, 48]. In the USA, a higher

Country	Origin	Prevalence (%)	Assay	Reference [No.]
Argentina	Sows	37.80	MAT	Venturini et al. (2004) [6]
Brazil	Farm	25.50	In-house ELISA	de Sousa et al. (2014) [7]
	Slaughter	19.50	IFAT	Cademartori et al. (2014) [8]
	Indoor raised	11.50	In-house ELISA	Luciano et al. (2011) [9]
	Free range	20.60		
	Farm	13.40	MAT	Piassa et al. (2010) [10]
Canada	Finisher	0.74	ELISA kit[4]	Poljak et al. (2008) [11]
Chile	Slaughter	8.80	ELISA kit[4]	Munoz-Zanzi et al. (2012) [12]
China	Finishers	4.60	IHAT	Chang et al. (2013) [13]
	Farm	29.60	In-house ELISA	Du et al. (2012) [14]
	Slaughter	12.00	IHAT	Wu et al. (2012) [15]
	Finishers	24.50	In-house ELISA	Tao et al. (2011) [16]
	Mixed farm	53.40	ELISA kit[5]	Yu et al. (2011) [17]
	Sows	14.40	IHAT	Huang et al. (2010) [18]
Czech Republic	Slaughter	36.00	ELISA kit[2]	Bartova and Sedlak (2011) [19]
France	Slaughter	2.00	ELISA kit[3]	Roqueplo et al. (2011) [20]
Germany	Finisher	4.10	In-house ELISA	de Buhr et al. (2008) [21]
	Sows	16.50	IFAT	Damriyasa et al. (2004) [22]
Ireland	Finishers	4.70	LAT	Halova et al. (2013) [23]
Italy	Indoor raised	16.10	IFAT, 1/16	Veronesi et al. (2011) [24]
	Slaughter	16.30	ELISA kit[1]	Villari et al. (2009) [25]
Latvia	Finishers	0.40	In-house ELISA	Deksne and Kirjusina (2013) [26]
	Free ranged	6.20		
Malaysia	Sows	0.00	IFAT	Chandrawathani et al. (2008) [27]
Mexico	Backyard	17.20	MAT	Alvarado-Esquivel et al. (2012) [28]
	Farm	0.50		
Nepal	Slaughter	11.70	In-house ELISA	Devleesshouwer et al. (2013) [29]
Panama	Indoor raised	32.10	IFAT	Correa et al. (2008) [30]
Peru	Slaughter	27.70	Western blot	Saavedra et al. (2004) [31]
Poland	Slaughter	26.40	MAT	Sroka et al. (2008) [32]
Portugal	Farm	9.80	MAT	Lopes et al. (2013) [33]
Romania	Backyard	30.50	IFAT	Pastiu et al. (2013) [34]
	Sows	12.40		
	Finishers	0.00		

Country	Origin	Prevalence (%)	Assay	Reference [No.]
Serbia	Slaughter	9.20	MAT	Klun et al. (2011) [35]
Slovakia	Slaughter	2.16	ELISA kit[1]	Turcekowa et al. (2013) [36]
	Sows	4.26		
Spain	Finisher	9.70	MAT	Garcia-Bocanegra et al. (2010) [37]
	Sows	24.20		
Switzerland	Finishers	14.00	ELISA kit[3]	Berger-Schoch et al. (2011) [38]
	Adult	3.60		
	Free range	13.00		
Taiwan	Slaughter	10.10	LAT	Tsai et al. (2007) [39]
The Netherlands	Organic	10.9	In-house ELISA	Kijlstra et al. (2008) [40]
	Indoor raised	0.40		van der Giessen et al. (2007) [41]
	Organic	2.70		
	Free range	5.60		
	Organic	3.00	ELISA-kit[3]	Meerburg et al. (2006) [42]
	Indoor raised	0.00	LAT	Kijlstra et al. (2004) [43]
	Free range	4.70		
	Organic	1.20		
USA	Indoor raised	2.60	ELISA kit[4]	Hill et al. (2010) [44]
	Outdoor raised	6.80	In-house ELISA	Gebreyes et al. (2008) [45]
	Indoor raised	1.10		
	Free range	25.00	ELISA kit[4]	Dubey et al. (2008) [46]
	Slaughter	16.40	Western blot	Saavedra et al. (2004) [31]
Vietnam	Finisher	23.00	MAT	Huong et al. (2007) [47]
	Sows	32.30		
	Free range	35.70		

Adapted from Guo et al. [48].

[1]Institut Pourquier, Montpellier, France; [2]ID Screen Toxoplasmosis Indirect, ID-Vet, Grabels, France; [3]p30-ELISA; [4] SafePath Laboratories, Carlsbad, CA; [5]Haitai Biological Pharmaceuticals Co., Ltd., Zhuhai, People's Republic of China.

Table 1. Global seroprevalence of *T. gondii* in domestic pigs.

(25%) prevalence was detected in the free range animals than in the outdoor- (6.8%) or indoor-ranged finishers (2.8%) [46, 51]. In the Netherlands, a spectacular drop was seen for the finishers (from 54 to 1.9%; Ref. [72]) and for the seropositive sows (30.9–5.6%; Ref. [41]). In the most industrialized countries, the prevalence is not higher than 5% [2]. Nevertheless, the low prevalence cannot however, prevent the high transmission rate to humans, as even as low as 1% is equal to 1 million *T. gondii* seropositive animals, infecting potentially 300 million

humans [52]. To date, there is no obligatory screening or notification system for the detection of seropositive animals on farms or in slaughterhouses within the EU-states. These observations and the possible reasons of the decreased prevalence are discussed in detail under Section 5 (Risk factors associated with porcine toxoplasmosis).

3. Genetic diversity

Because of the high clinical relevance, the parasite has been isolated from many naturally infected animals and humans, and molecular analysis of the parasitic genome was performed using restriction fragment length polymorphism (RFLP), PCR, or random amplified polymorphism DNA [53]. The obtained data led to genetic identification of the isolated parasites; and therefore, allowed to distinguish three major multilocus genotypes of *T. gondii*. The genetic lines named I, II, and III correspond to the genetic analysis of the polymorphic surface antigen 2 locus (*SAG2*) [54] and are characterized by a different degree of virulence and pathogenicity in LD50 in mice. Type I is considered as the most virulent genotype, followed by type III and type II. The latter is the most prevalent type present in animal reservoir and human population throughout Europe. Additionally, next to the typical three lineages, some of the strains obtained from South America, Africa, and Asia show an atypical genome or a mixed genotype of the existing ones. These strains are often characterized by an increased virulence in mice [2, 55]. Concerning the genotypes of pigs' isolates, the majority of the strains circulating within the porcine population in North America belong to the genotypes II and III or has a mixed allele composition, while in Europe, genotype II seems to be the most predominant clonal type [56, 57]. In South America, on the contrary, genotype I or mixed types are prevalent in domestic pigs [58].

4. Immune responses and tissue distribution upon acute and chronic toxoplasmosis

Upon infection with the parasite, the humoral and cellular immune responses are initiated, while clinically toxoplasmosis in pigs proceeds mainly asymptomatic. Only occasionally, pigs develop clinical signs. Following experimental inoculation of pigs with any infectious stage of *T. gondii* (oocysts, tissue cysts, or tachyzoites), a range of antibodies develops against the parasitic antigens. The time interval between the inoculation and the first detection of the immunoglobulins or cytokines varies depending on the virulence of the used strain, the infectious stage, and the applied dose [37, 59–62].

Secondly, a significant Th1-immune response can be observed as an increase of IFN-γ production after inoculation of pigs with a well-defined number of the infectious *T. gondii* strains [61, 63–65]. This increase is positively correlated with the duration of the experiment and can be detected in the serum collected from the infected animals in the supernatant from cultured PBMCs, and also as IFN-γ mRNA expression in PBMCs and intestinal lymphoid tissues. The IFN-γ production associated with the induced toxoplasmosis in pigs was in this

case the result of the activation of the innate and acquired immune system. These immune responses were investigated *in vitro* by determining the cytokine profile until 14 [61], 40 [63], or 56 dpi [66].

Next to IFN-γ, other cytokines are involved in the immune response. The infection of pigs with the VEG-strain oocysts induced a Th-1 immune response shortly after the inoculation, with the production of IL-15 and TNF-α mRNA [61]. Subsequently, a Th-2 response profile with IL-10 as anti-inflammatory cytokine was detected after the acute phase of the infection, dominated by IFN-γ production [63].

During the acute phase of the infection, the immune responses are directed against the disseminating tachyzoites, while throughout the chronic toxoplasmosis they also target the latent cysts with bradyzoites in the variety of tissues. It is well described that the parasite can persist within the intermediate host for a life span and can be found in all internal organs and muscles [67–69]. For instance, in pigs, viable *T. gondii* was recovered from porcine brain, heart, tongue, diaphragm, liver, and kidneys. Additionally, all edible commercial cuts of meat tested are also positive, representing thereby, a potential risk for consumers [4, 70]. It is a subject of discussion and ongoing research whether the host can clear the tissues during the chronic infection phase. In pigs, there is a scientific evidence that tissue cysts can gradually decrease in number and show a decline in viability, as tested by bioassay [66].

5. Risk factors associated with the porcine toxoplasmosis

Several factors can potentially modify the risk of *T. gondii* infection in pigs, such as the presence of cats on farms, rodent control, age of the animals, size and type of the herd, outdoor access, the carcass disposal, and feeding of unprocessed animal products such as goat whey to the pigs [41, 44, 49, 71].

The cat is responsible for the direct transmission of toxoplasmosis to farm animals such as pigs by entering the stables and shedding the oocysts in the animal facilities, or by contaminating the environment, and indirect spread of oocyts by animals entering the stables like dogs or birds. The free access to stables for people, without the application of strict hygienic measures (e.g., disinfecting foot bath or protective footwear and clothes for the exclusive use in the animal facilities) can also contribute to the dissemination of the oocysts within the herd or its transmission to the stables from the contaminated environment. The lack of these measures is especially important if domesticated or feral cats live in the close neighborhood of the animal facilities.

The second factor, namely the insufficient rodent control in the stables drives the persistence of the parasite on farm level in two ways: the rodents serve as a constant reservoir of the parasite for the cats, maintaining directly the infection risk within the herd; additionally, the rodents can directly be involved in the infection transmission by the predation or accidental ingestion of the mice by pigs [40, 56].

Age as a risk factor is strictly associated with a longer exposure and evidenced by an increased seroprevalence in older animals in comparison with piglets. As colostral antibodies disappear by 120 days of age, piglets and young raising pigs do not have any maternal immune protection, and are, therefore, exposed to the infection [71]. However, the

humoral immunity alone is not sufficient in combating the parasite. Indeed, the antibodies facilitate the cytotoxicity by the innate immune cells toward the extracellular parasite, but the intracellular *T. gondii* can escape from that. The age-dependent increase in seroprevalence in pig farms have been described in numerous studies, showing a higher incidence in adult pigs (19.5%) than in young animals (10.9%) [28], or in breeding sows than in finishers (30.9% and 1.9%, respectively, in the Netherlands [72] and 24.2% and 9.7%, respectively, in Spain [28].

The age of the animals at slaughter has also an important implication for the transmission of the disease toward human consumers and the epidemiology of human toxoplasmosis. Indeed, the younger the animals, the more chance that the meat will be consumed fresh and unprocessed, while the meat derived from older animals such as sows, will undergo processing to different pork products, which is harmful for the parasite and thus, safer for the consumer in terms of parasitic load [49].

Two major factors contributing to porcine toxoplasmosis are the size of the herd and the management type of the herd with a reverse correlation between the size and the on-farm prevalence: small herds showed a higher rate of seropositive animals (4.1%) than medium (1.9%) or large (0.6%) herds [51]. The reason for that would be the higher exposure per animal in smaller farms due to the lower density of the pigs. Even more critical for the risk for toxoplasmosis is the management type of the farm. The recently observed decrease in seroprevalence of toxoplasmosis in the pig population in the developed countries might be due to the implementation of the modern management system in porcine herds, with a visible shift from housing of a smaller number of animals in less strictly confined establishments or outdoor, to large scale facilities with a high output and a fast turn-over, characterized by all-in-all-out or farrow-to-finish models [44, 48, 73]. The extensive pig production expressed by the increasing number and size of porcine herds is driven by the high consumption of porcine meat in the developed countries in Europe and in the USA. In Belgium for instance, the total yearly number of pigs comprise 6.5 million animals, housed in 5000 conventional farms, ranging between on average 700–1300 pigs, as estimated by the National Institute of the Calculations, Federal Public Service Economy, (Actualization of the Industrial Study on Pork, 2015). Consequently, nearly 12 million of animals are slaughtered each year, due to an excessive consumption rate per inhabitant (35 kg/year), in comparison with other countries.

Summarizing, in the modern large-scale herds, the risk of *T. gondii* infection and thus, the prevalence of seropositive animals can be substantially reduced by the employment of a strict confinement housing with restrictive biosecurity regulations [1, 44, 45, 48].

However, in the last years, a new tendency in animal husbandry deserves the attention, namely the animal-friendly herds, housing organic or free range animals, providing daily a permanent or a temporary outdoor access to the animals. The term 'organic' refers to the quality and safety of the porcine products, with constraints about the chemical compounds originating from the feed or drug treatment, while 'free range' stands for the life quality of the animals during the production round. Hence, organic pigs are mainly reared outdoor, receive an organic feed, are provided with an animal friendly living space, the piglets are weaned at the later age than 3–4 weeks as on the intensive farms, and undergo a restrictive use of the antibiotics. The free range pigs differ from the regular pigs by the outdoor access and straw bedding, but are fed with a standard porcine feed and may receive drug treatment, if necessary, without losing a label

as in case of the organic pigs [43]. In both types of farming, pigs are continuously exposed to the parasite by contact with contaminated soil or ground water, and can easily transmit the infection further in the food chain [43, 44, 74].

As indicated above, an appropriate rodent control is of significant importance for the reduction of the risk for porcine toxoplasmosis; in addition to the latter, a proper carcass disposal seems to be equally essential, since both measures are intended to avoid the ingestion of formerly infected tissues [44, 56]. The cases of cannibalism by the accidental access to preliminary dead animals, especially when animal tissues are buried or composted are considerably common [41, 44, 49, 71]. Similarly, providing drink water of unknown quality to the pigs, possibly contaminated with the oocysts and feeding of raw animal products such as goat whey is also a potential risk, if made from unprocessed milk containing tachyzoites from a recently infected animal undergoing the dissemination phase [42, 75].

Finally, the prevalence of porcine toxoplasmosis may be influenced by the climate and geographical factors, for example, in altitude, temperature, and humidity, since they have a direct impact on the survival of *T. gondii* oocysts in the environment. As established previously, the oocysts remain infectious for a long period of time in a humid and cold environment, as long as they are not dried out or frozen. Hence, the prevalence of toxoplasmosis in pigs is higher in the mountains (32.1%) than in semi desert areas in Mexico (14%) [28], or in the coastal and northern regions of the USA (2.9–3.2%) than in the south continental part of the country [76].

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