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Toxoplasma gondii Infection in South-East Europe: Epidemiology and Epizootiology

Branko Bobić, Ivana Klun, Aleksandra Nikolić and Olgica Djurković-Djaković

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<http://dx.doi.org/10.5772/50831>

1. Introduction

Toxoplasmosis is a globally distributed parasitic zoonosis. Cats and other *Felidae* are the definitive host of the *Toxoplasma gondii* parasite, in whose intestines the sexual cycle takes place, and they are the primary source of infection to all animals including humans, by way of contaminating the environment with oocysts excreted in the feces (Dubey, 2010). Herbivores are infected by ingestion of food and water contaminated by oocysts, and carnivores by eating tissue cysts present in the flesh of infected animals. Omnivores including humans are infected by both routes – by oocysts via improperly washed vegetables or fruits, contaminated water or hands, and by tissue cysts via improperly processed or raw meat. Vertical transmission, from mother to offspring, may occur, and is the cause of congenital toxoplasmosis (CT). CT is actually the major *T. gondii*-induced clinical entity, which, along with opportunistic infection in immunocompromised patients, defines the clinical significance of toxoplasmosis. The gravity of the potential consequences of CT on the one hand, and the preventability of the disease on the other, call for implementation of prevention programs. A prerequisite for an adequate choice of prevention strategy is continuous monitoring of the local epidemiological situation.

This chapter reviews the epidemiology and epizootiology of *T. gondii* infection in South-East Europe (SEE). SEE is here considered as the territory comprising the Balkan Peninsula (35° – 46°53' N latitude, 13°23' – 30° E longitude), bordered by the Adriatic Sea to the west, the Mediterranean Sea to the south, the Black Sea to the east and the rivers Sava and Danube to the north, encompassing the countries descending from ex-Yugoslavia including Slovenia, Croatia, Bosnia & Herzegovina, Serbia, Montenegro and FYR of Macedonia (FYRoM), as well as Albania, Bulgaria and mainland Greece. Most of the area is mountainous, while the climate varies from Mediterranean to moderate. The whole region has a combined area of 550,000 km² and a population of 55 million.

2. Epidemiology of toxoplasmosis in SEE

We analysed epidemiological data published in the last 20 years for all SEE countries except Bulgaria and Bosnia & Herzegovina for which none were available. Data in the published reports were obtained using a wide array of immunodiagnostic assays which may somewhat limit comparisons. Indeed, the tests in use have varied over time both among and within individual countries, and included the Sabin-Feldman test (SFT), complement fixation test (CFT), indirect fluorescence (IFAT) to direct agglutination (DA) and ELISA, whether in-house or commercial; the latter ones were obtained from various manufacturers. However, this limitation applies to any such review (Gilbert & Peckham, 2002), and moreover, the pattern of infection observed in the region despite the variety of tests with their different specificities, sensitivities, cut-offs etc., rather emphasizes the described trends.

The vast majority of epidemiological data on toxoplasmosis in SEE comes from studies in women of generative age, and a few from studies in immunocompromised patients.

2.1. Toxoplasmosis in generative age women

Data on the prevalence of *T. gondii* infection in SEE countries are presented in Figure 1. In the last ten years, the prevalence has not surpassed 50% anywhere in the region, ranging from 20% in Greece (Diza et al., 2005) to 49% in Albania (Maggi et al., 2009). Wide differences in the prevalence of infection are generally characteristic of Europe, since the infection prevalence is currently ranging from 8.2% in Switzerland (Lausanne and Geneva) (Zufferey et al., 2007) to 57.6% in Timisoara-Romania (Olariu et al., 2008). Differences in the prevalence of *T. gondii* infection are commonly explained by differences in life-style habits pertaining to risk factors for transmission in particular milieus. Not many studies on infection risk factors have been published in SEE countries, but those available identified consumption of undercooked meat as the leading risk factor for transmission in Serbia and, more recently, in Albania (Bobić et al., 1998; 2003; 2007; Maggi et al., 2009), and contact with soil in northern Greece and FYRoM (Decavalas et al., 1990; Diza et al., 2005; Cvetković et al., 2010). Exposure to soil was also considered to account for the higher prevalence of infection in rural *vs.* urban women in Croatia.

Continuous monitoring of the prevalence of *T. gondii* infection in women of childbearing age in Slovenia, Serbia and Greece has showed a significant decrease in the infection prevalence since the eighties onwards. The largest decrease, from 86% in 1988 to 31% in 2007 (Bobić et al., 2003; 2007), was noted in Serbia. Furthermore, a trend of decreasing prevalence has been shown during the last decade in FYRoM, from 25% in 2002 to 20% in 2005 (Cvetković et al., 2003; 2010) and in Montenegro, a rather dramatic one, from 41% in 2001 to 27% in 2007 (Mišković et al., 2003; Rajković & Vratnica, 2008). These data suggest that a decrease in the prevalence is a region-wide feature (Fig. 1).

A decreasing trend of *T. gondii* infection prevalence noted in the SEE region is obviously part of a Europe-wide changing pattern of *T. gondii* infection over the last 30 years (Aspöck & Pollak, 1992; Nowakowska et al., 2006; Berger et al., 2009). Many factors have contributed

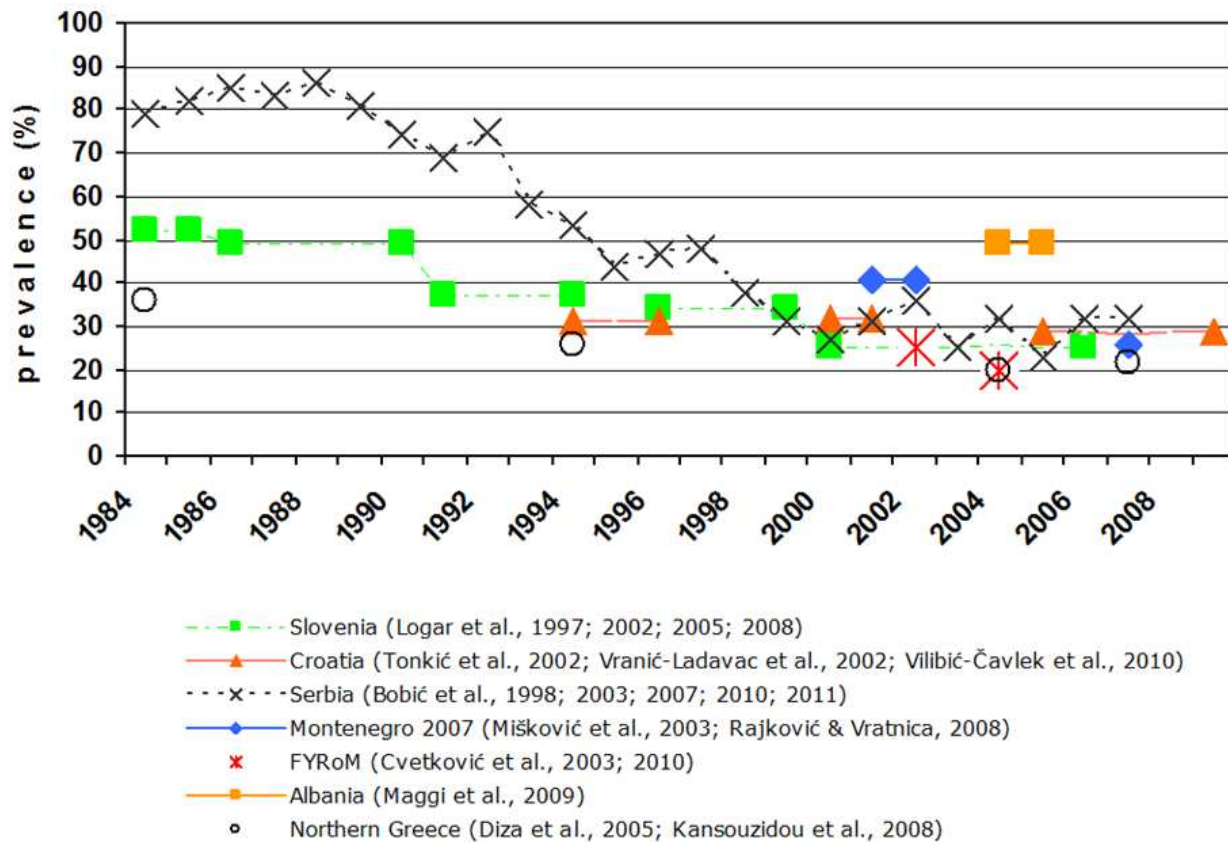


Figure 1. Prevalence of *Toxoplasma gondii* infection in generative age women in South-East Europe countries (1984-2009)

to such a change, including increased public awareness as a result of health education, better hygiene on livestock farms, and more frequent use of frozen meat. However, according to the SEE data, the listed factors seem not to be exhaustive; although better farming conditions along with the increased consumption of frozen meat (freezers now present in most households) may have contributed to a decrease in the infection prevalence in Serbia and Albania, a decreasing trend was also noted in FYRoM and Greece where consumption of undercooked meat was not found to be a risk factor.

It appears that, according to reports from the eastern part of the region, there is a north-to-south decrease in the infection prevalence (Table 1). For instance, in 1994, the prevalence ranged from 69% in southern Hungary (as a region neighbouring the SEE to the north) (Szénási et al., 1997), over 53% in Serbia (Bobić et al., 2003) to 26% in northern Greece (Diza et al., 2005). This trend was also evident within SEE in all years for which comparative data were available (2002: Serbia 36%, FYRoM 25%; 2004: Serbia 32%, Northern Greece 20%; 2007: Serbia 31%, Northern Greece 21%) (Cvetković et al., 2003; Diza et al., 2005; Bobić et al., 2007; Kansouzidou et al., 2008; Bobić et al., 2011). Moreover, a significant north-to-south decrease in the infection prevalence was also shown within Serbia itself (Bobić et al., 2003). The north-to-south decrease in the prevalence of infection within SEE suggests a possible influence of climatic conditions, which vary across the region from continental to Mediterranean, and over time as well.

Year	South Hungary	Serbia	FYR Macedonia	Northern Greece
1991	73% ⁽¹⁾	69% ⁽²⁾		
1992	70% ⁽¹⁾	75% ⁽²⁾		
1993	64% ⁽¹⁾	58% ⁽²⁾		
1994	69% ⁽¹⁾	53% ⁽²⁾		26% ⁽⁶⁾
2002		36% ⁽³⁾	25% ⁽⁴⁾	
2004		32% ⁽³⁾	20% ⁽⁵⁾	20% ⁽⁶⁾
2007		31% ⁽⁸⁾		21% ⁽⁷⁾

1) Szénási et al., 1997; 2) Bobić et al., 1997; 3) Bobić et al., 2007; 4) Cvetković et al., 2003; 5) Cvetković et al., 2010; 6) Diza et al., 2005; 7) Kansouzidou et al., 2008; 8) Bobić et al., 2011

Table 1. Decrease of prevalence of *Toxoplasma gondii* infection from North to South in three SEE countries

Seasonality of infection was examined in Slovenia (Logar et al., 2005) in the west and Serbia (Bobić et al., 2010) in the east. Both studies showed a strong seasonality, with significantly more cases of acute infection in the winter than in the summer months (Fig. 2). In Slovenia, seasonality of infection was attributed to "more frequent and closer contacts with potentially *T. gondii* infected cats, which prefer to stay indoors during this period" (Logar et al., 2005). In contrast, more cases of acute infection in the winter in Serbia were explained by a higher influence of undercooked meat consumption in the winter period (Bobić et al., 2010).

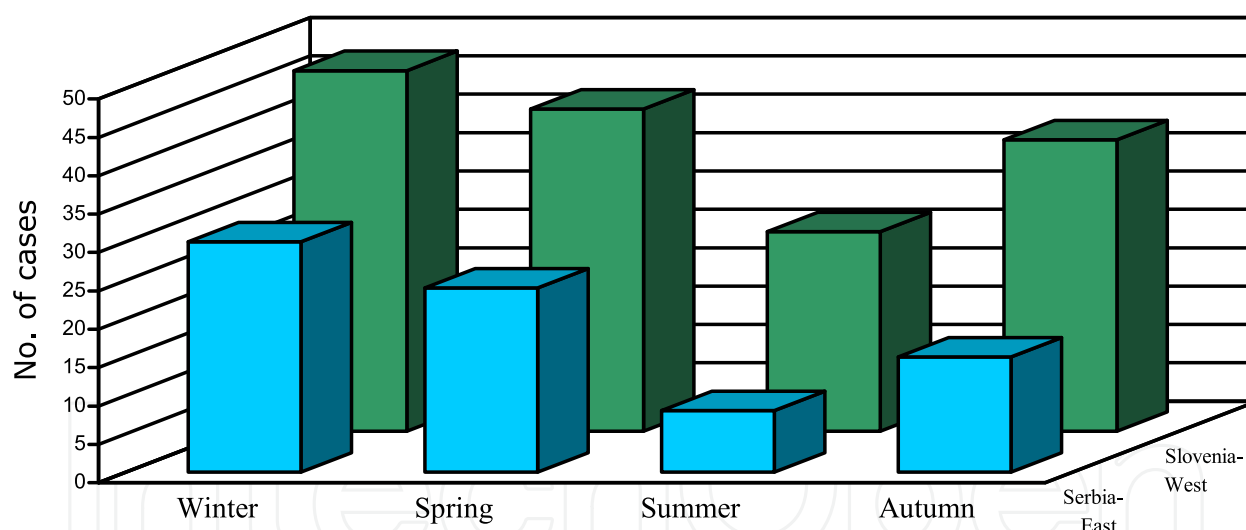


Figure 2. Seasonality of *Toxoplasma gondii* infection in Slovenia (1999-2004) and Serbia (2004-2008) (Logar et al., 2005; Bobić et al., 2010)

Accordingly, the decrease in the prevalence of *T. gondii* infection in the SEE seems independent of the varying influence of risk factors for infection transmission throughout the region. However, this may prove not to be entirely true, as the above analysis was based on the limited data reported, which were often not acquired through systematic nation-wide research, and which were mainly derived from epidemiological questionnaires of variable precision level. For an accurate insight into the risk factors of major significance for human *T. gondii* infection, more research, based on larger patient series and carried out in different SEE areas, including case-control studies, would be preferable.

2.2. Toxoplasmosis in immunocompromised patients

Toxoplasmosis is a major opportunistic infection causing life-threatening disease in immunocompromised individuals, which is considered to be a consequence of reactivation of previously latent infection. Although opportunistic infection due to *T. gondii* has long been known in organ/tissue transplantations and patients with malign or systemic diseases on treatment with immunosuppressive effect, data on toxoplasmosis in the immunocompromised population in SEE are available only from groups of HIV-infected individuals and even these are scarce. In Serbia, out of a cohort of 339 patients diagnosed with AIDS during a five year period (1991-95), 288 were tested for *T. gondii* infection and the prevalence was 44.1% (Djurković-Djaković et al., 1997). A more recent Croatian study of 219 blood donors and 166 HIV-infected patients referred to the Zagreb University Hospital for Infectious Diseases "Dr. Fran Mihaljević" in 2000-2001, the seroprevalence of toxoplasmosis was 52.5% and 51.8%, respectively (Đaković-Rode et al., 2010), confirming that the prevalence of *T. gondii* infection among HIV-positive individuals is similar to that in the general population. Interestingly, whereas the risk for developing TE was not associated with age, sex or HIV transmission risk factor in Serbia (Djurković-Djaković et al., 1997), the Croatian study established a higher risk of *T. gondii* infection (OR 2.37) for men who have sex with men (Đaković-Rode et al., 2010). In the Serbian study, of the 288 examined HIV-infected patients, 31 developed toxoplasmic encephalitis, indicating an overall attack rate of 7.8%. At the time, the cumulative incidence of toxoplasmic encephalitis in *T. gondii*-seropositive patients was estimated at 32.7% for 60 months. However, the subsequent wide use of highly active antiretroviral treatment has allowed for good control of formerly AIDS-defining opportunistic infections including toxoplasmosis, significantly decreasing their significance.

2.3. Molecular epidemiology

Although the population structure of *T. gondii* isolates throughout the world is currently a major research interest in the field of toxoplasmosis (Ajzenberg et al. 2002; Sibley et al., 2009), such studies are at its beginning in SEE. As concerns the data on *T. gondii* genotypes present in the SEE region, there is a single published report of a type II strain genotype isolated from a case of congenital toxoplasmosis in Serbia (Djurković-Djaković et al. 2006). Current data on this issue in Serbia are reported elsewhere in this book (Ivović et al.). It is to be hoped that with the increased use of molecular methodologies in the region, work will be performed which will contribute to the pan-European map of *T. gondii* genotypes.

2.4. Prevention of human toxoplasmosis

Strategies for the prevention of CT include general screening-in-pregnancy programs and health education, and countries with a low prevalence of infection generally opt for health education (Kravetz et Federman, 2005; Gilbert and Peckham, 2002), while those with a high prevalence adopt screening-in-pregnancy programs (Aspöck & Pollak, 1992; Thulliez, 1992). In the SEE, a systematic program for the prevention of CT based on serological screening of pregnant women and health education has been implemented in Slovenia, while no other country has a systematic prevention program (Logar et al, 2002). The changing pattern of

infection across the region currently complicates the choice of prevention strategy. A decrease in the prevalence of infection in women of childbearing age implies a rising proportion of women susceptible to infection in pregnancy, which, in turn, may lead to an increase in the incidence of congenital infections. Indeed, an increase in the incidence of primary infections in pregnancy has been shown through systematic screening-in-pregnancy programs; in Austria, which has been screening all pregnant women ever since 1975, the decrease in *T. gondii* prevalence from 50% to 37% during the 1980s was followed by an increase in the incidence of primary infections in pregnancy from 0.4% to 0.8% (Aspöck & Pollak, 1992). Similarly, in Slovenia, the decrease in the prevalence of infection from the early 1980s onwards, therefore before the introduction of the systematic prevention program in 1995, has been associated with an increased incidence of infections in pregnancy from 0.33% in the early 1980s to 0.75% in the early 1990s and to 0.94% in the late 1990s (Logar et al., 1995; 2002).

The decreasing prevalence of *T. gondii* infection and a possible subsequent increase in the incidence of primary infections in pregnancy warrants introduction of CT prevention programs in the SEE countries. A prerequisite for such programs to be cost-effective, however, is an accurate assessment of the proportion of women of childbearing age susceptible to infection in pregnancy and the subsequent incidence of CT. These data are not yet available for most of the SEE, and are further complicated by the changing pattern of infection across the region. Thus, a sound and financially sustainable alternative that may be recommended for all of the SEE, similar to what has been recommended for Serbia (Bobić et al., 2003), includes health education of all women of childbearing age focusing on the locally significant risk factors for infection transmission in particular countries.

Health education is an adequate preventive measure for uninfected immunocompromised patients too.

3. Epizootiology of toxoplasmosis in SEE

Epizootiological surveillance of *T. gondii* infection in meat animals is important both as a measure essential for animal health and economics of livestock production, and for assessment of the risk for human health, as infected meat animals represent an important reservoir for human infection.

Throughout SEE, few data are available on *T. gondii* infection in animals and most are not the result of systematic research. Early reports, in particular from Serbia/Yu, dealt with isolation of viable *T. gondii* from various species. By bioassay of brains and/or hearts in ground squirrels (*Spermophilus citellus*) *T. gondii* was isolated from 0.25% chickens (Simitch et al., 1961); and 0.7% pigs, 0% sheep and cattle, 6.2% guinea fowl, 3.8% turkeys, 3.6% ducks, 1% geese and 0% pigeons (Simić et al., 1967). By mouse bioassay, *T. gondii* has been isolated from pork, and from pig and sheep diaphragms in Croatia/Yu (Wikerhauser et al., 1983; 1988). In Bosnia/Yu, Živković and Arežina (1991) reported apparent *T. gondii* in smears from muscle tissues of farmed layers.

The data on strain isolation in animals are scarce; researchers in Bulgaria have reported isolation of both virulent and avirulent strains of *T. gondii* from domestic and wild animals,

but did not perform genetic typing (Arnaudov, 1978; Arnaudov et al., 2003; Arnaudov & Arnaudov, 2005). In Serbia, genotyping of *T. gondii* strains isolated from animals (sheep, pigs, pigeons) is in progress, and the first results confirm the dominance of type II strains (unpublished data).

Earlier serological investigations of various animal species in SEE countries showed a prevalence of 37% for cattle, 30% for sheep, 26% for pigs, 17% for horses, 41% for dogs, 25% for cats, 52% for house mice and 20% for rats in Serbia, but except for pigs, they were carried out on samples of limited size (Šibalić, 1977). In Bulgaria, Nankov (1968) has shown a 15.7% prevalence in hares, and Arnaudov (1971, 1973) reported a prevalence of 32.6% for sheep and 27.2% for goats.

An overview of studies of *T. gondii* infection in animals in SEE countries in the last 20 years is presented in Table 2. Evidently, data are not available for a half of the SEE countries.

As in humans, and similar to the rest of Europe and elsewhere (Tenter et al., 2000; Hall et al., 2001), data on the prevalence in animals vary quite widely in SEE. The highest prevalence has been reported for cattle, sheep and goats (Table 2). While farming practices are similar throughout the SEE region, differences within the region mostly occur in the climatic conditions and terrain characteristics.

The single nation-wide survey on *T. gondii* infection in meat animals in SEE, performed recently in Serbia, showed high seroprevalence rates of 76.3% in cattle and 84.5% in sheep and a lower one, of 28.9%, in pigs (Klun et al., 2006). This study showed that cattle from Western Serbia were at an increased risk of infection compared to all other regions (Table 3), possibly associated with a comparably increased humidity in this region (Klun et al., 2006). The levels of specific antibody determined in the cattle were relatively low (not above 1:400),

Country	Species	No. examined	% Positive	Test (cut-off)	Origin of animals	Ref.
Croatia	Goats	79	13.9	MAT (1:20)	Farms	Rajković-Janje et al., 1993
	Goats	100	4	MAT (1:20)	Farms	
	Σ Goats	179	8.4	MAT (1:20)	Σ	
	Sheep	unknown	11.6 (sheep) 9.4 (lambs)	DAT	Farms	Rajković-Janje et al., 1994
	Sheep	unknown	0.5	DAT	Nomadic	
	Σ Sheep	334	4.8	DAT	Σ	
	Sheep	unknown	48.4	ELISA	10 farms	Marinculić et al., 1997
	Chickens	716	0.4	Bioassay	Abattoir	Kutičić & Wikerhauser, 2000
	Rats	142	1.4	Bioassay	Pig farms	Kutičić et al., 2005
	Mice	86	0	Bioassay	Pig farms + households	

Serbia	Cattle	611	76.3	MAT (1:25)	Farms + abattoir	Klun et al., 2006
	Sheep	511	84.5	MAT (1:25)	Farms	
	Pigs	605	28.9	MAT (1:25)	Farms + abattoir	
	Horses	250	30.8	MAT (1:25)	Farms	Klun, 2005
	Sheep	367	7.1	unknown	Aborting sheep	Vidić et al., 2007
	Sheep	30	36.7	IFAT (1:20)	Farm	Lalošević et al., 2008
	Pigs	488	9.2	MAT (1:25)	Abattoir	Klun et al., 2011
	Goats	356	74.7	MAT (1:25)	Farms	Djokić et al., 2011
	Rats	80	27.5	MAT (1:25)		
	Rats	144	7.6	Microscopy		
	Mice	12	3	Microscopy	Urban	Vujanić et al., 2011
	Rats	144	10.4	Real-time PCR		
	Mice	12	83.3	Real-time PCR		
	Pigeons	30	13.3	MAT (1:25)	Urban (wild)	Personal observations
Bulgaria	Wild animals (7 species)	643	0 – 16.7	Agar gel micro precipitation	Wild	Arnaudov et al., 2003
	Sheep	380	48.2	IHAT (1:10)		
	Goats	364	59.8	IHAT (1:10)	Farms	Prelezov et al., 2008
Greece (mainland)	Sheep	840	53.4	IFAT		
	Sheep	450	58.5	ELISA	Farms	Kontos et al., 2001
	Cattle	105	20	ELISA	Dairy farms	Diakou et al., 2005a
	Sheep	350	52.6			
	Goats	280	62.9	ELISA	Mixed stock farms	Diakou et al., 2005b
	Sheep	184/182	52.2/50.5	ELISA (a)		
	Goats	229/167	22.3/18	ELISA (a)	Organic farms	Ntafis et al., 2007
	Horses/equids	753/773	1.7/1.8	ELISA (a)	Farms	Kouam et al., 2010
	Dogs	2512	31.8	ELISA	unknown	Haralabidis & Diakou, 1999
	Pigeons	379	5.8	ELISA	Domestic flocks	
	Pigeons	50	0	ELISA	Urban (wild)	Diakou et al., 2011
	Σ Pigeons	429	5.1	ELISA	Σ	

(a) Mean OD_{negative controls} + 3SD**Table 2.** *Toxoplasma gondii* infection in animals in SEE countries in the last 20 years

which is consistent with reports on the rapid decrease of *T. gondii* antibody in cattle (Dubey et al., 1985). Cattle are relatively resistant to *T. gondii* infection (Dubey and Thulliez, 1993), but it is unclear whether this is associated with fast elimination of cysts from cattle tissues (Dubey, 1983), or there is inconsistent cyst formation following infection. In addition to farm location in the western region, the only other risk factor determined for cattle infection was small herd size. According to the type of housing, the results showed that access to outside pens was protective as compared to total confinement. This apparently paradoxical finding may represent a further argument for the importance of the way in which feed is kept, or possibly indicates involvement of other farm factors not identified in this type of study.

The same study showed a prevalence of 85% in sheep in Serbia, of which 10% had high antibody levels of $\geq 1:1600$, suggestive of acute infection. Although arbitrary, the cut-off of 1:1600 is even conservative since Dubey and Welcome (1988) had considered a titre of 1:1024 high. However, correlation with ovine abortions could not be established, since etiological laboratory diagnosis of ovine abortions in Serbia does not include diagnosis of *T. gondii*. Regionwise, similarly as with cattle, sheep from Western Serbia were at an increased risk of infection as compared to all other regions (Table 3). An increased risk of infection was also found in state-owned vs. private large flocks. Compared to other SEE countries, the prevalence of 85% is quite high; in Croatia and Bulgaria the highest recorded prevalence in sheep is 48%, and in Greece 58.5% (Table 2). This is also evident in goats; compared with the prevalence of 74.7% established in Serbia (Djokić et al., 2011), it was lower in Greece (62.9%) (Diakou et al., 2005b) and Bulgaria (59.8%) (Prelezov et al., 2008), and very markedly so in Croatia – 8.4% (Rajković-Janje et al., 1993).

An outbreak of toxoplasmosis in sheep has recently been reported; massive abortions (60%) occurred in a flock of 500 dairy sheep in Northern Greece at 110-130 days of pregnancy, diagnosed upon observation of tissue cysts in brain smears of aborted fetuses, and by serological (ELISA) examinations of mother and fetal serum samples. The abortion rate declined immediately upon instituting sulfadimidine therapy (Giadinis et al., 2011).

In horses, who generally have lower seroprevalence values than small ruminants (van Knapen et al., 1982), a prevalence of 30.8% has been determined in a study in Serbia (Klun, 2005), vs. only 1.7% in Greece (Kouam et al., 2010).

In pigs, an overall seroprevalence of 29% was established in Serbia (Klun et al., 2006). Of those seropositive, 4% were likely to be in the acute stage of infection, indicating continuous presence of infection reservoirs in the environment. Risk factors included age and farm type (Table 3). Since pigs are continuously exposed to infection, the increase in the risk of infection with age, ranging from 15% in market weight age pigs to 41% in adults, was expected, and repeated previous findings (Dubey et al., 1991; Dubey et al., 1995; Weigel et al., 1995; Damriyasa et al., 2004). Pigs on finishing type farms were four-fold more likely to be infected than those from farrow-to-finish farms. According to these results, it was proposed that a national strategy to reduce the level of *T. gondii* infection in pigs should include a shift towards the development of more farrow-to-finish farms, as well as vigilance in farm management and implementation of zoo-hygienic measures at finishing farms. Damriyasa et al. (2004) stated that *T. gondii* seropositivity is an indicator of the hygienic status of the pig farm.

Species	Factor	Adjusted OR	95% CI	P-value
Cattle	Herd size			
	Large (n>100)	1.00		
	Small (n<10)	2.19	1.28–3.75	0.004
	Type of housing			
	Stable only	1.00		
	Access to outside pens	0.37	0.21–0.67	0.001
	Access to pasture	0.72	0.25–2.07	0.548
	Region			
	Northern Serbia	1.00		
	Western Serbia	2.04	1.10–3.79	0.024
Sheep	Central/Eastern Serbia	0.97	0.32–2.90	0.951
	Belgrade District	0.60	0.30–1.20	0.148
	Farm type			
	Private large (n>100)	1.00		
	State-owned (n>100)	4.18	2.18–8.00	<0.001
	Private small (n<10)	1.79	0.55–5.81	0.332
	Region			
	Northern Serbia	1.00		
	Western Serbia	4.66	1.18–18.32	0.028
	Central/Eastern Serbia	0.82	0.25–2.73	0.748
Pigs	Age group			
	Market weight (<8 months)	1.00		
	Adult (≥8 months)	3.87	2.60–5.76	<0.001
	Farm type			
	Farrow-to-finish	1.00		
	Finishing	3.96	1.97–7.94	<0.001

Table 3. Risk factors for *T. gondii* infection in meat animals in Serbia in 2003. Final logistic regression models. Results presented as adjusted odds ratio (OR) and 95% confidence intervals (CI) (modified from Klun et al., 2006)

A recent study on *T. gondii* infection in slaughter pigs in Serbia (Klun et al., 2011) showed, however, a three-fold lower prevalence of 9.2% in a total of 488 swine from abattoirs in the vicinity of Belgrade. This difference was largely attributed to the difference in the studied samples since the latter one consisted of a large majority (96%) of market-weight pigs, who generally have a much lower prevalence than adult pigs. Similarly to the 2006 study, risk factors for infection in slaughter pigs included age and farm type with a 41-fold higher likelihood of infection in adult *vs.* market-weight pigs ($p<0.001$), and a 15-fold higher likelihood of infection in pigs of all ages from smallholders' finishing type farms ($p<0.001$) *vs.* those from farrow-to-finish intensive farms.

On the other hand, the modern approach in farm management to provide for the welfare of the animals as well as organic food for human consumption is to develop animal-friendly (organic) farms. According to experiences from the Netherlands (Kijlstra et al., 2004), development of such farms may result in an increase in *T. gondii* infection. Nevertheless, a single report from organic sheep and goat farms in Greece (Ntafis et al., 2007) showed similar prevalence rates to those in animals from conventionally managed farms (Kontos et al., 2001; Diakou et al., 2005b).

A major reason for the control of *T. gondii* infection in meat animals is the reduction of the reservoir of human infection. Cattle are generally thought not to be significant in this context (Dubey and Thulliez, 1993). However, beef is often consumed undercooked ('rare' beef steaks, roast beef, steak tartar), and at least one outbreak of toxoplasmosis whose source was raw beef has been documented (Smith, 1993). In addition, one out of four beef samples randomly chosen from UK retail outlets tested positive for *T. gondii* by PCR (Aspinall et al., 2002). These facts, along with the circumstantial evidence provided by the data on the high prevalence of cattle infection of 92% in Italy and 69% in France (see Tenter et al., 2000), and now in Serbia, countries in which human infection is highly prevalent as well, all suggest a role for cattle as a *T. gondii* reservoir for human infection. In addition, Bobić et al. (2007) have demonstrated that among all the meat consumed, undercooked beef presents the highest risk for human infection in Serbia. Similarly, although Opsteegh et al. (2011a) did not establish a correlation between seropositivity and the detection of parasites in cattle, a study in which the relative contribution of sheep, beef and pork products to human *T. gondii* infection in the Netherlands was quantified (by Quantitative Microbial Risk Assessment), showed that beef is indeed an important source even if the seroprevalence in cattle is low (Opsteegh et al., 2011b).

On the other hand, according to official statistical reports (RZS, 2006–2010), pork represents approximately 50% of all meat consumed in Serbia. Thus, although pigs were the least infected of the examined species, given the findings that the prevalence increases with age and reaches 41% in sows (Klun et al., 2006), pork consumption may significantly contribute to human infection. When used for cooking, pork is generally properly thermally processed, but in most of the SEE countries' tradition mature pork is also highly valued for making delicatessen meat products. Raw or improperly cured sausages and ham are the source of small (family) epidemics of trichinellosis which, in spite of mandatory meat examination for

Trichinella spiralis, occasionally occur in Serbia (Djordjević 1989, Čuperlović et al., 2005), and thus, are a quite plausible source of human *T. gondii* infection as well.

For most meat animals, although a trend is generally (worldwide) difficult to establish due to the scarcity of studies in most countries (no two time points), there is no visible reduction in the prevalence of *T. gondii* infection, as opposed to the decreasing trend in humans, discussed in detail earlier in this chapter (and explained by reasons including increased frozen meat use, better farm management etc.). For the most part, farming practices and environmental contamination have not changed, and except for the intensive pig farms in which a major reduction in *T. gondii* prevalence has occurred, a decline in the prevalence of *T. gondii* infection in meat animals is yet to be achieved. Moreover, for strictly herbivorous species that require outdoor access, this is probably impossible (Kijlstra & Jongert, 2009).

Ubiquitous contamination of the environment is also evident from the presence of *T. gondii* in both farm and urban rodents (Kutičić et al., 2005; Vujanić et al., 2011), wild animals, and pigeons (Arnaudov et al., 2003; Arnaudov & Arnaudov, 2005; Diakou et al., 2011) (Table 2). In dogs, studies performed in Greece have shown a prevalence ranging from 21.2% to 30.8% (Chambouris et al., 1989), and of 31.8%, in a large series of 2512 dogs from the regions of Macedonia and Thrace (Haralabidis & Diakou, 1999). As long as there is evidence of such widespread environmental contamination in SEE, a change for the better and a decrease of *T. gondii* infection in meat animals may hardly be expected in the absence of energetic and systematic prevention measures throughout the region.

4. Conclusion

Existing strategies for the prevention of toxoplasmosis in countries which have been implementing them for years have led to a decrease in its incidence, but have not solved the problem of congenital infection. This clearly shows that new comprehensive strategies for the prevention of toxoplasmosis are needed. These should be based on accurate and validated data on (1) the routes and risk factors for human infection on local level, which will allow for a more efficient health education; (2) routes and risk factors for meat animal infection to diminish infection reservoirs; (3) environmental contamination. Epidemiological and epizootiological data presented in this chapter show how far along this road we have come, and more importantly, how far we still have to go to achieve successful prevention of *T. gondii* infection in the SEE region.

Author details

Branko Bobić, Ivana Klun, Aleksandra Nikolić and Olgica Djurković-Djaković*

National Reference Laboratory for Toxoplasmosis, Centre for Parasitic Zoonoses (Centre of Excellence in Biomedicine), Institute for Medical Research, University of Belgrade, Serbia

* Corresponding Author

Acknowledgement

This work has been supported by project grant No. III 41019 from the Ministry of Education and Science of Serbia.

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