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Toxoplasmosis in Animals in the Czech Republic – The Last 10 Years

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1. Introduction

Toxoplasmosis is a significant zoonosis that affects humans and warm blooded animals. The definitive hosts of parasite *Toxoplasma gondii* are cats and other felids. Many species of domestic, wild or zoo animals may serve as intermediate hosts.

In humans, clinical form of toxoplasmosis is rare in immunocompetent people, while it may leads to eye diseases, CNS or generalized infection in immunocompromised individuals as well as interfere with the course or outcome of pregnancy. In Europe, *T. gondii* seroprevalence in humans ranges from 8% to 77% (Dubey 2010). In the Czech Republic, *T. gondii* antibodies were detected in 35% and 25% pregnant women by Sabin-Feldman Test (SFT) and Complement Fixation Test (CFT), respectively (Hejlicek et al. 1999). Repeated prevalence studies in humans in some European countries (France, Belgium, Sweden and Norway), revealed an evident trend of a decrease in *T. gondii* seroprevalence (Welton and Ades 2005). The same trend is observed in the Czech Republic. The prevalence of infection varies among ethnic groups due to sanitary and cooking habits. Consumption of raw or almost raw, dried, cured or smoked meat from domestic animals, unpasteurized goat milk or consumption of meat from wild animals may be associated with ingestion of the parasite (Kijlstra and Jongert 2008, Jones et al. 2009). Higher prevalence was found also in people who had frequent contact with animals and soil, such as abattoir workers, garbage handlers and waste pickers (Dubey and Beattie 1988). Children playing with dogs and cats can be infected by direct contact because animals can act as mechanical vectors (Etheredge et al. 2004).

In animals, *T. gondii* infection is a frequent cause of early embryonic death and resorption, fetal death and mummification, abortion, still birth and neonatal death. Thus, toxoplasmosis in domestic and farm animals is a disease of great importance for veterinary medicine and husbandry since it can cause productive and economic losses.

T. gondii antibodies have been found in animals worldwide. Seroprevalence to *T. gondii* varies among countries, within different areas of a country and within the same city. Dubey (2010) summarized the results of seroprevalence studies performed on different groups of animals from several countries.

In the Czech Republic, some important studies concerning *T. gondii* in animals were done in past years. The seroprevalence of *T. gondii* infection in domestic animals obtained by different serological methods is summarized in Table 1.

Animal	Prevalence	Assay	Reference
Cat	17 – 91%	SFT, CFT, MPA, IFAT	Havlik and Hubner 1958, Zastera et al. 1966, Zastera et al. 1969, Svoboda and Svobodova 1987, Svoboda 1988
Dog	15 – 58%	SFT, CFT, MPA	Havlik and Hubner 1958, Zastera et al. 1966, Zastera et al. 1969, Svoboda and Svobodova 1987, Hejlíček et al. 1995, Hejlíček et al. 1995
Sheep	4 – 77%	SFT, CFT, MPA, IHA, IFAT	Zastera et al. 1966, Zastera et al. 1969, Arnaudov et al. 1976, Kozojed et al. 1977, Hejlíček and Literak 1994c
Goat	20 – 86%	SFT, CFT, IFAT	Havlik and Hubner 1958, Zastera et al. 1966, Zastera et al. 1969, Hejlíček and Literak 1994b, Literak et al. 1995, Slosarkova et al. 1999
Cattle	2 – 42%	SFT	Havlik and Hubner 1958, Zastera et al. 1969, Kozojed et al. 1977
Pig	0.1 – 38%	SFT, CFT, MPA	Havlik and Hubner 1958, Zastera et al. 1969, Kozojed et al. 1977, Hejlíček and Literak 1993, Hejlíček and Literak 1994b, Vostalova et al. 2000
Horse	4 – 11%	SFT, CFT	Havlik and Hubner 1958, Zastera et al. 1969, Kozojed et al. 1977, Hejlíček and Literak 1994a, Zastera et al. 1966
Gallinaceous bird	0 – 20%	SFT	Zastera et al. 1965, Zastera et al. 1969, Kozojed et al. 1977, Literak and Hejlíček 1993
Water fowl	2 – 33%	SFT	Zastera et al. 1965, Zastera et al. 1969, Literak and Hejlíček 1993
Rabbit	6 – 95%	SFT, CFT	Havlik and Hubner 1958, Havlik and Hubner 1960, Zastera et al. 1969, Kunstýr et al. 1970, Hejlíček and Literak 1994d

SFT – Sabin-Feldman Test, CFT – Complement Fixation Test, MPA – Microprecipitation in Agar, IHA – Indirect Hemagglutination Assay, IFAT – Indirect Fluorescent Antibody Test

Table 1. *Toxoplasma gondii* prevalence and assays used in different groups of domestic animals in the Czech Republic until year 2000

In a group of game animals, a prevalence of 15% was found in wild boars by SFT (Hejlíček et al. 1997), 4 – 31% in hares by SFT or Microprecipitation in Agar (MPA) (Havlik and Hubner 1958, Zastera et al. 1966, Vosta et al. 1981, Hejlíček et al. 1997), and 14 – 58% prevalence in wild ruminants by SFT (Havlik and Hubner 1958, Zastera et al. 1966, Hejlíček et al. 1997).

These studies were performed by one or by a combination of methods such as SFT, CFT, MPA and Indirect Hemagglutination Assay (IHA). Nowadays, these methods are less frequently used; it is preferred to use Modified Agglutination Test (MAT), and/or Indirect Fluorescent Antibody Test (IFAT), and/or an Enzyme-Linked Immunosorbent Assay (ELISA), and/or a Latex Agglutination Test (LAT). This trend is also evident from a recent review summarized worldwide prevalence of *T. gondii* infection in animals and humans (Dubey 2010).

Based on the results of examination of different groups of animals in the State Veterinary Institute Prague in years 2003 – 2006, it is evident that lethal toxoplasmosis in the Czech Republic is the most important in some species of zoo animals; while in domestic animals it was not proved (Sedlak and Bartova 2007). Contrary, the sera of cats and dogs were the most frequently examined. Insufficient attention is paid to small ruminants that can abort or have reproduction disorders due to toxoplasmosis with subsequent economic losses.

That is why during the last 10 years, our research team focused on *T. gondii* serosurveys in different groups of animals to obtain actual data and to evaluate which group of animals is the most affected by *T. gondii* infection. Following parts of chapter summarises the results of seroprevalence studies in domestic, game and zoo animals tested by using IFAT, ELISA and LAT with the possibility to compare the results with those obtained from other countries with the same methods used. The results of experimental studies and cases of clinical toxoplasmosis recorded in the Czech Republic are mentioned too.

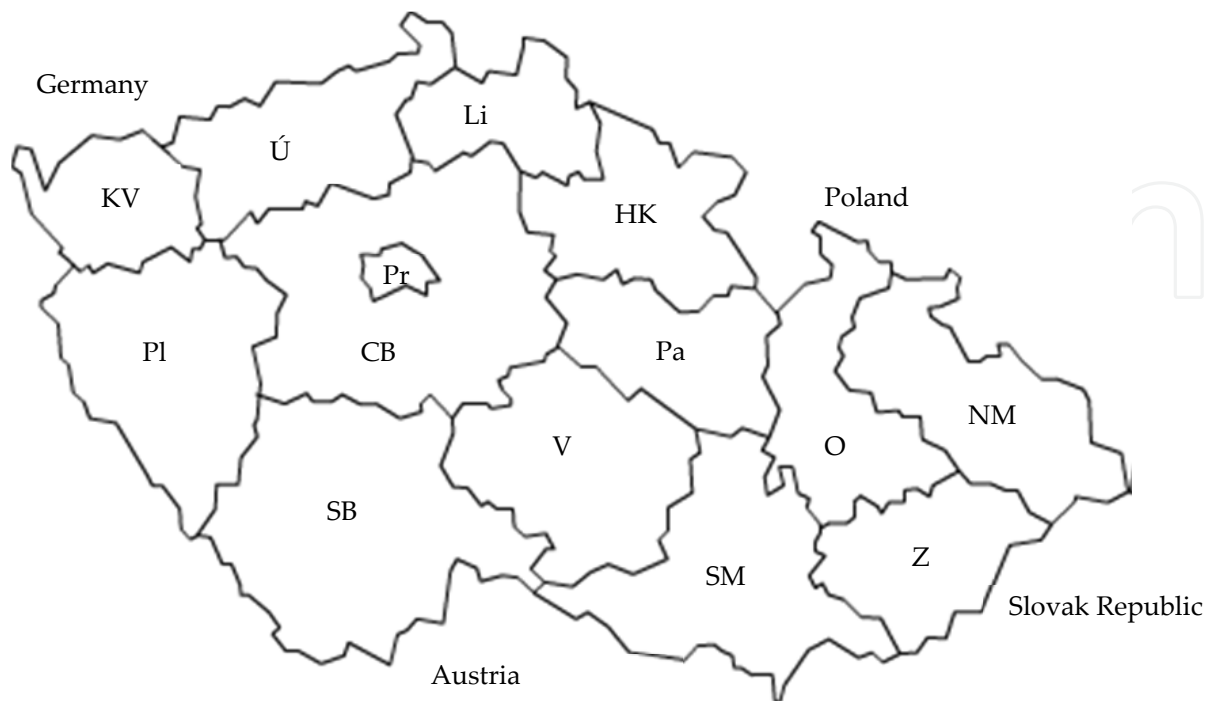
2. Toxoplasmosis in domestic animals

2.1. Recent data from the Czech Republic

Serological studies

During years 1995-2012, the samples of blood were collected from different groups of animals and examined for specific *T. gondii* antibodies. The animals tested for *T. gondii* antibodies were clinically healthy, no case of abortion or other symptoms of toxoplasmosis were recorded. The blood samples were collected by veterinarians on farms, zoo or during hunting seasons and sent to State Veterinary Institute Prague for routine examination.

In a group of domestic animals, in total 4254 animals were tested with the following number of animals used: 286 cats, 413 dogs, 547 sheep, 251 goats, 546 cattle, 551 pigs, 552 horses and 1108 poultry (217 chickens and 293 broilers, 60 turkeys, 178 geese and 360 ducks). The animals came from 2 – 14 different districts of the Czech Republic (Figure 1).



CB – Central Bohemia (cat, dog, sheep, goat, cattle, pig, horse, poultry, wild boar, hare, wild ruminant), HK – Hradec Králové (goat, cattle, pig, horse, poultry, wild boar), KV – Karlovy Vary (goat, cattle, pig, horse, poultry, wild boar, wild ruminant), Li – Liberec (goat, cattle, pig, horse, poultry, wild boar, wild ruminant), Pa – Pardubice (goat, horse, poultry), Pl – Plzeň (goat, cattle, pigs, horse, poultry, wild boar, wild ruminant), Pr – Prague (goat, cattle, horse, poultry, wild ruminant), O – Olomouc (poultry, hare, wild ruminant), NM – North Moravia (cat, dog, poultry, wild ruminant), SB – South Bohemia (horse, pig, poultry, wild boar, wild ruminant), SM – South Moravia (poultry, hare, wild ruminant), Ú – Ústí nad Labem (sheep, goat, cattle, pig, horse, poultry, wild boar, wild ruminant), V – Vysočina (pig, poultry, wild ruminant), Z – Zlín (poultry)

Figure 1. Map of the Czech Republic showing the sampled area with domestic and game animals tested for *T. gondii* antibodies.

Sera of domestic animals were tested for *T. gondii* antibodies by an indirect fluorescent antibody test (IFAT), using the Sevatest Toxoplasma Antigen IFR (Sevac, Prague, Czech Republic) and specific conjugates, by an ELISA (Institut Pourquier, Montpellier, France), or by a latex agglutination test (Pastorex TM Toxo, Biorad, France). The data on the method and cut-off used, specific conjugate for IFAT and producer are summarized in Table 2.

In a group of domestic animals, *T. gondii* antibodies were found in 66% goats, 59% sheep, 44% cats, 36% pigs, 26% dogs, 23% horses, 12% poultry (43% goose, 14% ducks, and 0.3% in broiler; turkeys and chickens were negative) and 9.7% cattle. The results of serological examination including the number of samples tested, the method and cut-off used, the number and percentage of positive samples, titres or %S/P obtained in positive samples and reference about published data are summarized in Table 3.

Animal	Assay (cut-off)	Conjugate for IFAT	Producer
Cat	IFAT (≥ 40)	anti-cat IgG	Sigma Aldrich, USA
Dog	IFAT (≥ 40)	anti-dog IgG	Sigma Aldrich, USA
Sheep	ELISA ($\geq 50\%$ S/P)	–	Sigma Aldrich, USA
Goat	ELISA ($\geq 50\%$ S/P)	–	Sigma Aldrich, USA
Cattle	ELISA ($\geq 50\%$ S/P)	–	Sigma Aldrich, USA
Pig	ELISA ($\geq 50\%$ S/P)	–	Sigma Aldrich, USA
Horse	LAT	anti-horse IgG	VMRD, Pulman, USA
Chicken Broiler	IFAT (≥ 40)	anti-chicken IgG	Sigma Aldrich, USA
Turkey	IFAT (≥ 40)	anti-chicken IgG	Sigma Aldrich, USA
Goose	IFAT (≥ 40)	anti-duck IgG	KPL, USA
Duck	IFAT (≥ 40)	anti-duck IgG	KPL, USA

IFAT – Indirect Fluorescent Antibody Test, ELISA – Enzyme-Linked Immunosorbent Assay, LAT – Latex Agglutination Test

Table 2. Serologic method, cut-off, specific conjugates for IFAT and producer used in domestic animals.

Animals	<i>T. gondii</i>			Assay (cut-off)	Titres or %S/P	Reference
	n	positive	%			
Cat	286	126	44	IFAT (40)	40 – 81920	Sedlak and Bartova 2006b
Dog	413	107	26	IFAT (40)	40 – 10240	Sedlak and Bartova 2006b
Sheep	547	325	59	ELISA (50%S/P)	50 – 200	Bartova et al. 2009a
Goat	251	166	66	ELISA (50%S/P)	56 – 191	Bartova et al. 2012
Cattle	546	53	9.7	ELISA (50%S/P)	50 – 200	Bartova et al. (unpublished)
Pig	551	198	36	ELISA (50%S/P)	50 – 337	Bartova and Sedlak 2011
Horse	552	125	23	LAT	–	Bartova et al. 2010a
Poultry						
Chicken	510	0	0	IFAT (40)	–	Bartova et al. 2009a
Broiler	293	1	0.3	IFAT (40)	40	Bartova et al. 2009a
Turkey	60	0	0	IFAT (40)	–	Bartova et al. 2009a
Goose	178	77	43	IFAT (40)	40 – 2560	Bartova et al. 2009a
Duck	360	52	14	IFAT (40)	40 – 320	Bartova et al. 2009a

IFAT – Indirect Fluorescent Antibody Test, ELISA – Enzyme-Linked Immunosorbent Assay, LAT – Latex Agglutination Test

Table 3. The result of serological examination of domestic animals with method used, cut-off, titres and %S/P in positive samples

Experimental studies

In the Czech Republic, two experimental studies were conducted on domestic poultry.

The first study was conducted on chickens (*Gallus domesticus*) that were inoculated *per os* with two different doses of *T. gondii* oocysts (Sedlak et al. 2000b). Antibodies to *T. gondii* were detected by IFAT first on day 14 p.i.; all chickens were serologically positive on days 21 and 28 p.i. No clinical symptoms were recorded. Parasite *T. gondii* was isolated from heart, muscle, spleen and brain. In one case, no *T. gondii* was isolated from any organ. Based on this experiment chickens seems highly resistant to *T. gondii* infection.

The second experimental study was conducted on domestic ducks (Bartova et al. 2004). Ducks were inoculated *per os* with different doses of *T. gondii* oocysts. Antibodies to *T. gondii* were detected in all ducks by IFAT on day 7 p.i. Antibody titres were found in the range of 20–640 depending on the infectious dose of the oocysts. From day 14 p.i., antibody titres increased to 80–20 480. Bioassay in mice revealed *T. gondii* in the breast and leg muscles, heart, brain, liver and stomach. The infected ducks showed no clinical symptoms, however, the results of bioassay indicate that, compared to gallinaceous birds, domestic ducks are relatively susceptible to *T. gondii* infection.

2.2. Comparison of data obtained

Cats

Clinical signs of toxoplasmosis in cats include fever, anorexia, dyspnea, uveitis, pneumonitis and others. Kittens can develop acute toxoplasmosis and die from it. The seropositivity increases with the age of cat, indicating postnatal transmission of infection. *T. gondii* antibodies have been found worldwide (Dubey 2010). Seroprevalence varies among countries, within different areas of a country and within the same city. In Europe, the highest prevalence 76% was found by SFT in Turkey (Karatepe et al. 2008), while the lowest 17% in Israel by ELISA (Salant and Spira 2004). In the Czech Republic, we found 44% prevalence by IFAT. In the previous studies from the Czech Republic, 17% – 91% prevalence was found by SFT, CFT and MPA. During the last 20 years, there is a trend of decreasing seroprevalence especially in cats staying at home and fed with commercial diet.

Dogs

T. gondii antibodies have been found in canine sera worldwide. Seroprevalence increases with age indicating postnatal infection, is higher in dogs from rural areas, in dogs housed exclusively outdoors, in dogs eating birds, small mammals, meat, viscera and home-cooked meals (Lopes et al. 2011b). In Europe, the highest prevalence 75% was found by SFT in Turkey (Aktas et al. 1998), while the lowest 5% in Sweden by ELISA (Lunden et al. 2002). In the Czech Republic, we found 26% prevalence by IFAT. In the previous studies from the Czech Republic, 4% – 58% prevalence was found by SFT or CFT. The lower prevalence is recorded in dogs staying at home and fed with commercial diet.

Sheep

T. gondii has been recognized as one of the main cause of infective ovine abortion in New Zealand, Australia, the United Kingdom, Norway and the United States. In the Czech

Republic, not yet a case of toxoplasmic abortion has been recorded in sheep herds. Seroprevalence was shown to increase with age, suggesting that animals acquire infection postnatally, however transplacental transmission of *T. gondii* may be more common than previously believed. Antibodies to *T. gondii* have been found in sheep worldwide (Dubey 2010). There is no validation of any serological test for the detection of *T. gondii* infection in sheep; different methods and cut-off are used.

In Europe, the highest prevalence 96% was found by ELISA in Turkey (Mor and Arslan 2007), while the lowest 10% was found in Slovak Republic by SFT (Kovacova 1993). We found 59% prevalence by ELISA. In the Czech Republic, 4% – 77% prevalence was found in past years.

Based on experimental studies, *T. gondii* was more frequently detected in brain and heart than in muscles; however *T. gondii* was detected also in milk (Camossi et al. 2011). Attention should be paid to meat or milk consumed without sufficient temperature treatment.

Goats

T. gondii antibodies have been found in goats worldwide (Dubey 2010). In Europe, the highest prevalence 91% was found by LAT in Netherland (McSporran et al. 1985), while no antibodies were found in Poland by IFAT (Gerecki et al. 2005). We found 66% prevalence by ELISA. In the Czech Republic, 20% – 86% prevalence was found in past years.

Goats appear to be more susceptible to clinical toxoplasmosis compared to other domestic animals, and even adult goats could die of acute toxoplasmosis. In the Czech Republic, toxoplasmosis was diagnosed in two Angora goat herds in South Moravia with an outbreak of abortions and births of weak kids; the goats showed also iodine deficiency (Slosarkova et al. 1999). Based on several experimental studies conducted on goats, *T. gondii* was detected in liver, muscles, heart, diaphragm, brain, kidneys and could be excreted in semen and milk. Attention should be paid to raw goat meat and milk if consumed without sufficient temperature treatment.

Cattle

Serum antibodies to *T. gondii* have been found in cattle in many surveys worldwide (Dubey 2010). In Europe, the highest seroprevalence 92% was found by MAT in Italy (Avezza et al. 1993), while no antibodies were found in Slovak Republic (Pleva et al. 1997) and Turkey (Oz et al. 1995). Actual prevalence rates are likely to be lower than indicated because of problem with the specificity of the tests used. The SFT test gives false or erratic results with cattle sera; on the other hand a titer of 1:100 or higher in the MAT appears to be indicative of *T. gondii* infection in cattle (Dubey 2010). We found 9.7% seroprevalence by ELISA. In the previous studies in the Czech Republic, 2% – 42% seroprevalence was found by SFT and DT.

There are no confirmed reports of clinical toxoplasmosis in adult cattle. In cattle, *T. gondii* can be transplacentally transmitted resulting in abortions; but it is probably a rare occurrence. There is more important parasite *Neospora caninum* leading to abortion in cattle. In the Czech Republic, there is very low prevalence of *N. caninum* in herds of cattle. The ingestion of beef

or dairy products is not considered important in the epidemiology of *T. gondii* because cattle are not a good host for this parasite. Attempts to isolate *T. gondii* from cattle tissues have been unsuccessful, that is why it does not present risk of infection for humans.

Pigs

Clinical manifestation of toxoplasmosis in pigs could include diarrhea, encephalitis, pneumonitis, necrotic hepatitis and abortion. Surveys based on the presence of *T. gondii* antibodies in blood sera of pigs have been reported worldwide (Dubey 2010). In Europe, *T. gondii* prevalence declined in the last decade especially because of good management system. There is a different sensitivity and specificity of the assays used for serosurveys in the following order MAT, IHA, LAT and ELISA starting with the most sensitive one. Good correlation was obtained between ELISA and MAT. In Europe, the highest prevalence 64% was found by IFAT in Italy (Genchi et al. 1991), while only 1% prevalence was found by the same method used in Austria (Edelhofer 1994). In the Czech Republic, we found 36% prevalence by ELISA. In the previous studies from the Czech Republic, 0 – 38% prevalence was found by SFT, CFT or MPA.

The higher prevalence is found among pigs from small backyard operations, while the prevalence among pigs from traditional large farms and modern large-scale farms is usually lower. Attention should be paid if pork meat is consumed nearly raw or without sufficient temperature treatment.

Horses

Horses have been shown to be susceptible to *Toxoplasma* infection (Tassi 2006) however there is no confirmed report of clinical toxoplasmosis. Serum antibodies to *T. gondii* have been found in horses in many surveys worldwide (Dubey 2010). In Europe, the highest prevalence 37% was found by SFT in Turkey (Gazayagci et al. 2011), while the lowest 1% in Sweden by DAT (Jakubek et al. 2006). In the Czech Republic, we found 23% by LAT. In the previous studies from the Czech Republic, 4 – 11% prevalence was found by SFT or CFT.

By reason that equine meat represents an important source of food in many human communities, infected equine meat could represent potential risk of *T. gondii* infection for humans.

Poultry

In general, there is a different sensitivity of birds to *T. gondii* infection. Owls and other predatory birds and domestic poultry seem to be resistant to *T. gondii* infection, while e.g. rock partridge (*Alectoris graeca*), pigeons and canaries are highly susceptible to toxoplasmosis. In Europe, there were some reports of birds (galliformes, columbiformes, psittaciformes and passeriformes) that died due to toxoplasmosis (Dubey 2010). Toxoplasmosis can also lead to drop in egg production and high mortality in embryonated eggs. In the Czech Republic, confirmed clinical toxoplasmosis has not been recorded in birds. Little is known concerning the validity of the serologic tests for the detection of *T. gondii* antibodies in avian sera. It is preferred to use MAT, nevertheless other methods such as SFT, CFT, ELISA and IFAT have been used worldwide.

We found higher prevalence in water fowls (43% and 14% in goose and ducks, respectively) compared to gallinaceous poultry (0.3% in broiler; turkeys and chickens were negative). In Europe, higher prevalence 36% was found in chicken from Austria by MAT (Dubey et al. 2005), or 20% in turkeys by ELISA in Germany (Koethe et al. 2011).

T. gondii have been isolated from brain, heart and leg muscles, but not from the pectoral muscle and liver (Dubey et al. 1993).

3. Toxoplasmosis in game animals

3.1. Recent data from the Czech Republic

Serological studies

In majority of game animals, the course of infection is subclinical. However, considering the high prevalence of *T. gondii* infection in game animals, they should be taken into account as the possible source of infection for human.

A total of 1618 game animals were tested, including 720 wild ruminants or ruminants living in reservations (377 red deer, 79 roe deer, 14 sika, 143 fallow deer, 105 mouflon and 2 reindeer), 565 wild boars and 333 hares. The animals came from 3 – 11 districts of the Czech Republic (Figure 1).

Sera of game animals were tested for *T. gondii* antibodies by an IFAT, using the Sevastest Toxoplasma Antigen IFR (Sevac) and specific conjugates (Table 4). Sera with titer ≥ 40 were marked as positive.

Animal	Conjugate for IFAT	Producer
Wild boar	anti-swine Ig G	Sigma, Praha
Hare	anti-rabbit Ig G	Sigma Aldrich, USA
Red deer	anti-deer Ig G	KPL Inc. Maryland
Sika	anti-deer Ig G	KPL Inc. Maryland
Fallow deer	anti-deer Ig G	KPL Inc. Maryland
Roe deer	anti-deer Ig G	KPL Inc. Maryland
Mouflon	anti-goat Ig G	VMRD, USA
Reindeer	anti-deer Ig G	KPL Inc. Maryland

IFAT – Indirect Fluorescent Antibody Test

Table 4. Specific conjugates for IFAT and producer used in game animals.

In a group of game animals, *T. gondii* antibodies were detected in 32% wild ruminants (50% in sika, 45% red deer, 24% roe deer, 17% fallow deer, 9% mouflon, and in one reindeer), 26% wild boars and 20% hares. The results of serological examination including the number of samples tested, the method and cut-off used, the number and percentage of positive samples, titres obtained in positive samples and reference about published data are summarized in Table 5.

Animals	<i>T. gondii</i>			Assay (cut-off)	Titres	Reference
	n	positive	%			
Wild boar	565	148	26	IFAT (40)	40 – 1280	Bartova et al. 2006
Hares	333	71	20	IFAT (40)	40 – 640	Bartova et al. 2010b
Wild ruminants						
Red deer	377	169	45	IFAT (40)	40 – 640	Bartova et al. 2007
Roe deer	79	19	24	IFAT (40)	40 – 160	Bartova et al. 2008
Sika	14	7	50	IFAT (40)	80 – 320	Bartova et al. 2009
Fallow deer	143	24	17	IFAT (40)	40 – 160	Bartova et al. 2010
Mouflon	105	9	9	IFAT (40)	40 – 320	Bartova et al. 2011
Reindeer	2	1		IFAT (40)	80	Bartova et al. 2012

IFAT – Indirect Fluorescent Antibody Test, ELISA – Enzyme-Linked Immunosorbent Assay, LAT – Latex Agglutination Test

Table 5. The result of serological examination of game animals with the sample number, the method and cut-off used, titres in positive samples and references.

Experimental studies

In the Czech Republic, two experimental studies were conducted on game animals.

The first study was conducted on hares (Sedlak et al. 2000a). Hares were experimentally infected with *T. gondii* oocysts. Most infected hares demonstrated behavioural changes, and all of them died between 8 and 19 days. In all hares, parasitemia was demonstrated on days 7 and 12 p.i. *T. gondii* was isolated from liver, brain, spleen, kidney, lung, heart and skeletal muscles. Based on this result, hares seem to be very sensitive species to *T. gondii* infection.

The second study was conducted on gallinaceous game birds (Sedlak et al. 2000b). Partridges (*Perdix perdix*), chukars (*Alectoris chukar*), wild guineafowl (*Numida meleagris*) and wild turkeys (*Meleagris gallopavo*) were inoculated *per os* with two doses of *T. gondii* oocysts. Antibodies to *T. gondii* were detected in the birds by IFAT first on day 7 p.i. Two of five partridges fed 10^3 oocysts and six of eight partridges fed 10^5 oocysts died between day 6 and 16 p.i. No clinical symptoms were observed in surviving birds, however enteritis was the most striking lesion in partridges that died. Bioassay in mice revealed *T. gondii* in the brain, liver, spleen, heart and leg muscles of all partridges and chukars. These results indicate that partridges are highly susceptible to toxoplasmosis, while chukars, wild guineafowls and turkeys seem to be less susceptible.

3.2. Comparison of data obtained

Wild boars

In Europe, the highest seroprevalence 100% was found in wild boars from Portugal (Lopes et al. 2011b) or 44% in wild boars from Spain (Closa-Sebastia et al. 2011); while the lowest

prevalence 8% was found in wild boars from Slovak Republic (Antolova et al. 2007). In the Czech Republic we found 26% prevalence by IFAT. This prevalence was higher compared to 0% – 15% prevalence found by SFT in the previous studies from the Czech Republic.

The meat of wild boars may harbour tissue cysts of *T. gondii* and may represent a vehicle of human toxoplasmosis infection. Hejlicek et al. (1997) found tissue cysts in 2% examined wild boars from the Czech Republic, while in the neighbouring Slovakia, *T. gondii* was isolated from 31% of wild boars (Catar 1972). Hunters and their families consuming meat from wild boars should be aware of *T. gondii* infection and advised to take precautions. It is highly recommended to cook meat from wild boars thoroughly before human consumption.

Hares

There are several reports of *T. gondii* infection in hares from Europe (Dubey 2010). The highest seroprevalence 46% was found in hares from Germany (Frolich et al. 2003); in contrast no antibodies were detected in hares from Sweden (Gustafsson and Ugglå 1994). In the Czech Republic, we found 20% prevalence by IFAT. This result is comparable with 4% – 31% prevalence found in previous studies by SFT or MPA. Based on the results of experimental infection, hares seem to be sensitive to *T. gondii* infection; *T. gondii* was isolated from liver, brain, spleen, kidney, lung, heart and skeletal muscles (Sedlak et al. 2000).

Wild ruminants

T. gondii infection in game animals is of epidemiological significance. Deer are strictly herbivores and that is why the high prevalence of *T. gondii* in deer suggests widespread contamination of the environment with *T. gondii* oocysts. In red deer, the highest seroprevalence 32% was found by SFT in Scotland (Williamson and Williams 1980), while the lowest 8% by DAT in Norway (Vikoren et al. 2004). We found relatively high prevalence 45% by IFAT in red deer from the Czech Republic. In roe deer, the highest prevalence 63% was found in Norway and Sweden by SFT (Kapperud 1978), while the lowest 13% prevalence was found in Austria by IHA (Edelhofer et al. 1989). In the Czech Republic, we found 24% prevalence by IFAT that was also in range 14% – 58% prevalence found in our country in previous studies. In fallow deer, we found 17% prevalence that is comparable with 24% prevalence found in Spain by MAT (Gauss et al. 2006). In the Czech Republic, we found 9% prevalence in mouflon that is lower compared to 23% prevalence found in France (Aubert et al. 2010). In case of reindeer, only two animals were examined in the Czech Republic. This is very low number that is why it is not possible to compare it with 1% prevalence found in Norway by DAT (Vikoren et al. 2004).

Deer are popular game animals in several countries. The meat of deer may harbour tissue cysts of *T. gondii* and may represent a vehicle of human toxoplasmosis infection. Toxoplasmosis infection in men was documented after consumption of raw or nearly raw deer meat in USA (Sacks et al. 1983, Ross et al. 2001).

4. Toxoplasmosis in zoo animals

4.1. Recent data from the Czech Republic

Serological studies

In a group of zoo animals, 556 animals belonging to 114 species were tested (5 species of primates, 28 species of carnivores, 8 species of perissodactyla and 73 species of artiodactyla). The animals came from 12 zoo and 4 small private exotic centres in the Czech Republic.

Sera of zoo animals were tested for *T. gondii* antibodies by an IFAT, using the Sevatest Toxoplasma Antigen IFR (Sevac) and specific conjugates (Table 6). Sera with titer ≥ 40 were marked as positive.

Order and family	Indirect Fluorescent Antibody Test (IFAT)	
	Conjugate for IFAT	Producer of conjugate
Primates		
Cercopithecidae	anti-monkey IgG	Sigma-Aldrich s.r.o., Praha
Hominidae	anti-human IgG	Sevapharma, Praha
Carnivora		
Canidae	anti-dog IgG	Sigma-Aldrich s.r.o., Praha
Felidae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Hyaenidae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Mustelidae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Otariidae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Ursidae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Viveridae	anti-cat IgG	Sigma-Aldrich s.r.o., Praha
Perissodactyla		
Equidae	anti-horse IgG	VMRD, Pullman, USA
Artiodactyla		
Bovidae	anti-bovine IgG, anti-goat IgG	VMRD, Pullman, USA
Camelidae	anti-llama IgG	VMRD, Pullman, USA
Cervidae	anti-deer IgG	KPL, Gaithersburg, Maryland
Suidae	anti-swine IgG	Sigma-Aldrich s.r.o., Praha

Table 6. Specific conjugates for Indirect Fluorescent Antibody Test and their producer used in zoo animals.

In a group of zoo animals, *T. gondii* antibodies were detected in 193 of 556 (35%) animals, representing 72 of 114 species tested (Sedlak and Bartova, 2006a). According to order, *T. gondii* antibodies were found in 90% carnivorous, 45% primates, 33% perissodactyles and 22% artiodactyles. According to families, *T. gondii* antibodies were found in ursidae (100%), felidae (93%), canidae (88%), hominidae (73%), equidae (33%), suidae (29%), cervidae (27%), camelidae (26%), bovidae (20%), cercopithecidae (18%) and in 3 animals of hyeenidae, 2 animals of mustelidae and 2 animals of viveridae. The highest prevalence 100% was found

in Eurasian wolf (*Canis l. lupus*), Maned wolf (*Chrysocyon brachyurus*) and Sumatran tiger (*Panthera t. sumatrae*). The highest titre 40960 was found in Pallas's cat (*Otocolobus manul*). The results of serological examination of zoo animals are summarized in Table 7.

Order and Family	n	<i>T. gondii</i>	
		Positive	%
Primates	22	10	45
Cercopithecidae	11	2	18
Hominidae	11	8	73
Carnivora	87	78	90
Canidae	32	28	88
Felidae	41	38	93
Hyaenidae	3	3	
Mustelidae	2	2	
Otariidae	2	0	
Ursidae	5	5	100
Viveridae	2	2	
Perissodactyla			
Equidae	46	15	33
Artiodactyla	401	90	22
Bovidae	265	53	20
Camelidae	19	5	26
Cervidae	110	30	27
Suidae	7	2	29

Table 7. The result of serological examination of zoo animals

4.2. Experimental studies and cases of clinical toxoplasmosis

In the Czech Republic, experimental infection was conducted on budgerigars (*Melopsittacus undulatus*) that were orally inoculated with *T. gondii* oocysts with different doses (Kajerova et al. 2003). *T. gondii* antibodies were found by LAT in all birds. The birds showed no apparent signs of disease. *T. gondii* was isolated by bioassay in mice from all birds fed 10^3 or more oocysts. The results show that budgerigars are resistant to *T. gondii* infection.

Cases of clinical toxoplasmosis in the Czech Republic were recorded in nilgais (*Boselaphus tragocamelus*) and saiga antelope (*Saiga tatarica*) (Sedlak et al. 2004). Three captive female nilgais aborted two fetuses and two of their newborn calves died within two days of birth. Parasite *T. gondii* was demonstrated in the brains and livers of both fetuses and in one of the two neonates by single-stage polymerase chain reaction (PCR) with TGR1E and by semi-nested PCR with B1 gene. Retrospectively, antibodies titers ≥ 640 were found by IFAT in the sera of all three female nilgais and in one male nilgai used to breed them. Fatal toxoplasmosis was diagnosed in one captive adult female saiga antelope. Tissues cysts of *T. gondii* were found in the liver, lung, spleen, kidney, and intestine of saiga antelope. Toxoplasmosis was

confirmed also by PCR with TGR1E and immunohistochemically. Toxoplasmic hepatitis and pneumonia were considered to be a primary cause of death.

The other cases of fatal toxoplasmosis were recorded in year 2004 in seven Pallas cats in several zoos in the Czech Republic (Sedlak and Vodicka 2005).

4.3. Comparison of data obtained

There are many reports on toxoplasmosis in zoo animals. Marsupials, New World monkeys, hares and some small ruminants belong to the most sensitive to clinic toxoplasmosis. Fatal toxoplasmosis was also recorded e.g. in captive dik-dik and Pallas cats from zoo in USA (Riemann et al. 1974; Dubey et al. 2002), in lions from a zoo in Africa (Ocholi et al. 1989) and in a Siberian tiger from a zoo in Belgium (Dorny et al. 1989). In the Czech Republic, fatal toxoplasmosis was recorded in saiga and nilgais antelopes from Prague and Chomutov zoos (Sedlak et al. 2004) and in Pallas cats (Sedlak and Vodicka 2005).

In our study, antibodies to *T. gondii* were found in 90% carnivora, 45% of primates, 33% perissodactyla and 22% artiodactyla. When compared to other similar study concerning zoo animals, *T. gondii* antibodies were found in 47% carnivora, 25% artiodactyla and 23% primates (Gorman et al. 1986). We found 93% prevalence in felids; that is higher when compared with 32%, 64.9% or 75.8% prevalence found in felids from zoo in California (Riemann et al. 1974), Brazil (Silva et al. 2001) and Florida (Lappin et al. 1991), respectively.

The potential source of *T. gondii* infection for carnivores is meat contaminated with *T. gondii* tissue cysts; herbivores can be infected by food contaminated with *T. gondii* oocysts and omnivorous animals by both ways. To prevent spreading of *T. gondii* infection among zoo animals, cats, including all wild felids should be housed in buildings separated from other animals, particularly the most sensitive marsupials and New World monkeys. There must be protection against free access of domestic cats to sources of food and water or into the buildings with animals, especially those that are the most sensitive to toxoplasmosis. Feline faeces should be removed daily to prevent sporulation of oocysts.

5. Further research

Further work should focus on serological studies in other animal groups that are neglected but may represent a risk of infection for humans in case of consumption of their meat or other products. Such animals include, for example, rabbits, ostriches, pigeons, pheasants and mallard ducks. In addition, rodents, wild birds and wild carnivores (foxes, marten and others) may play an important part in the circulation of *T. gondii* infection in nature and thus represent a risk of infection for wildlife, domestic animals and human people alike. Serological studies should be supplemented with an evaluation of the infection risk factors and with the use of molecular methods to detect *T. gondii* in animal products, as well as to characterize *T. gondii* genotypes circulating in animal populations in the Czech Republic.

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