Epidemiological remarks on *Toxoplasma gondii* infection in Timișoara Zoo

Gheorghe Dărăbuș1, Mihăiţă Afrenie2, Rareş T. Olariu3, Marius S. Ilie1, Adrian Balint1, Ionela Hotea1

1 – Banat University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Department of Parasitology and Parasitic Diseases, Calea Aradului 119, Timişoara 300645, Romania.
2 – Zoological Garden, Pădurea Verde, strada Avram Imbroane 91, Timişoara 300377, Romania.
3 – “Victor Babeş” University of Medicine and Pharmacy, Piaţa Eftimie Murgu 2, Timişoara 300041, Romania.

Correspondence: Tel. +40256277251, Fax +40256277118, E-mail hotea_ionela@yahoo.com

Abstract. Between 2009 and 2010, 37 blood samples were collected from different animals species raised in captivity in the Timişoara Zoological Garden. These were: one domestic cat (*Felis catus*), three lions (*Panthera leo*), two wild cats (*Felis silvestris*), five goats (*Capra aegagrus*), three roe deer (*Capreolus capreolus*), one guanaco (*Lama guanicoe*), two reindeer (*Rangifer tarandus*), three horses (*Equus caballus*), four raccoons (*Procyon lotor*), one brown bear (*Ursus arctos*) and a rabbit (*Oryctolagus cuniculus*). The overall prevalence of *Toxoplasma gondii* infection confirmed by ELISA was 73.07%. All the carnivores and the omnivores tested positive for anti-*Toxoplasma* antibodies, thus exhibiting the highest prevalence of infection. 57.14% of the herbivores were found to be infected, whereas no *Toxoplasma* antibodies were detected in lagomorphs.

Keywords: *Toxoplasma gondii*; ELISA; Prevalence; Zoo animals.

Toxoplasmosis is one of the most common parasitic infections in humans and animals, estimated to rank third among the most widespread parasitic disease throughout the world (Cosoroabă, 2005; Dărăbuș et al., 2006; Hotea et al., 2008). It is a protozoosis caused by coccidia belonging to the Sarcocystidae family. *Toxoplasma gondii* infects various organs and tissues in over 350 species of vertebrates, being, from this point of view, probably the most polyxenous protozoa (Dărăbuș et al., 2006). *Toxoplasma gondii* has a heteroxenous biological life cycle and can virtually infect all species of warm-blooded animals (mammals and birds), including humans as intermediate hosts and felines as final hosts (Dubey et al., 2004; Dubey et al., 2007). The importance in human and veterinary medicine is proved by the parasite’s worldwide spread (Irabuena et al., 2004). Due to its zoonotic importance, *T. gondii* is the most studied of all the coccidia.

The disease has serious social and economic consequences as it causes spontaneous abortions, intrauterine deaths, birth defects, as well as psychiatric disorders in humans.
Humans, unlike animal intermediate hosts are not a source of infection for definitive hosts (Smith and Sherman, 2009).

In the feline definitive host, the disease usually evolves asymptotically. The cats’ habit of burying their faeces in grain warehouses favours the transmission of oocysts to farm animals. *T. gondii* oocysts can be dispersed by wind, rain, surface waters, etc. (Dubey, 2004), which makes this protozoosis particularly difficult to control. Toxoplasmosis, both in humans and in animals, has been studied extensively in Romania and globally (Tenter et al., 2000; Dubey et al., 2007; Hotea et al., 2008; Hotea et al., 2009). Serological testing (ELISA, IFAT, IHAT) has shown *T. gondii* to be particularly common among farm animals.

Detection of numerous cases of *T. gondii* infection in humans and in domestic animals abroad and in Romania, especially in the western and central regions of the country, coupled with a lack of national data on the prevalence of this parasitosis in wild animals raised in captivity, prompted a series of etiological and epidemiological investigations of animals in the Timișoara Zoo Garden.

**Materials and methods**

The study was carried out between 2009 and 2010 on 37 blood samples collected from wild or domestic animals raised in the Timișoara Zoo. The animal species we study were 6 carnivores, 14 herbivores, 5 omnivores and 1 lagomorph.

The blood samples were collected from animals of various ages, while the animals were under sedation for annual check-ups or occasional treatments. The samples were examined in the Parasitology and Parasitic Diseases Laboratory from the Faculty of Veterinary Medicine in Timișoara. The coproscopic examination of feces from definitive hosts (domestic and wild felines) was performed using standard flotation methods.

For the ELISA testing, the blood samples were collected in vacutainers. The serum was obtained through blood decantation and then, the latter was stored in a freezer until it was examined.

The serum samples were examined through indirect ELISA, using the 1D-VET Screen kits (ID VET, France) to determine the presence of *Toxoplasma*-specific IgG antibodies, resulting from infection with *T. gondii*. The 96-well plate is coated with P30 *T. gondii* antigen, and the antigen-antibody complex forms with the help of the peroxidase conjugate which is added later. For performing ELISA method we used the manufacturer instructions.

The optic densities (OD) obtained after the reading of the microplate were interpreted according to the following formula:

\[
\text{antibody titer} = \frac{\text{OD sample}}{\text{OD positive control}} \times 100
\]

Values above 200% were considered strongly positive, values between 50 and 200% were considered positive. Those between 40% and 50% were deemed uncertain, whereas values below 40% were considered negative.

**Results**

The prevalence of *T. gondii* infection in the animals tested by ELISA is shown in tables 1 and 2. The study has shown that 19 out of the 26 animals under study tested positive for *Toxoplasma* infection (73.07%). The evaluation of the prevalence of *Toxoplasma* infection in the zoo animals has highlighted the fact that carnivores are the most exposed to the *Toxoplasma* infection, followed by omnivores and herbivores.

**Table 1. The prevalence of *T. gondii* in animal species (intermediate hosts) raised in Timișoara Zoo**

<table>
<thead>
<tr>
<th>Species</th>
<th>Tested (n)</th>
<th>Positive (n)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capra aegagrus</td>
<td>5</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Capreolus capreolus</td>
<td>3</td>
<td>2</td>
<td>66%</td>
</tr>
<tr>
<td>Lama guanicoe</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Rangifer tarandus</td>
<td>2</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>Equus caballus</td>
<td>3</td>
<td>1</td>
<td>33%</td>
</tr>
<tr>
<td>Procyon lotor</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Ursus arctos</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Oryctolagus cuniculus</td>
<td>1</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>20</strong></td>
<td><strong>13</strong></td>
<td><strong>65%</strong></td>
</tr>
</tbody>
</table>
All of the carnivores tested, the domestic and the wild cats, as well as the lions, were found serologically positive for the *Toxoplasma* infection. Similarly, the 5 omnivores, the four raccoons and the bear, also tested positive. The 14 herbivores – ponies, goats, deers, guanaco – exhibited a relatively high prevalence of *T. gondii* infection, eight of them having anti-*Toxoplasma* antibodies (57.14%).

**Discussion**

The overall prevalence of *Toxoplasma gondii* infection confirmed by ELISA in the zoo animals under investigation was 73.07%. Carnivores are particularly vulnerable to *Toxoplasma* infection because of their diet, based exclusively on meat. The high rate of infection in omnivores can also be explained by the presence of meat in their daily diet. The presence of domestic cats in zoo premises, the caretakers’ free access to all the facilities, the way the animals are fed are all likely to favour the transmission of the infection.

The prevalence of *T. gondii* infection presented by other authors in certain wild animals, has been recorded as having values between 9.09% and 83.33% (Arnaudov et al., 2003; Dubey et al., 2004). The presence of toxoplasmosis in zoos is a managerial issue, because of the near impossible task of preventing felines from shedding oocysts on the premises on the one hand, and because of the free access of stray cats to the zoo grounds on the other. Competent management and implementation of adequate administrative programmes in zoos can be useful in preventing the transmission of toxoplasmosis to. Felines should not be fed raw meat which has not been previously frozen, because of the potential risk of infestation through tissue cysts. Meat should be first frozen and then defrosted, prior to being fed to the felines, since tissue cysts are destroyed by the low temperature (Tenter et al., 2000). The presence of stray cats in zoos needs to be carefully monitored because they are likely to shed oocysts. Species which are highly vulnerable to toxoplasmosis should be kept away from felines (Dubey et al., 2007).

Sporadically, cases of acute toxoplasmosis were reported in lions (*Panthera leo*), certain antelopes (*Madoqua guentheri*), meerkats (*Suricata suricatta*) and Mexican hairy porcupine (*Coendou mexicanus*) (Ocholi et al., 1989; Smith and Frenkel, 1995; Basso et al., 2009). Toxoplasmic abortions have been registered among female muskox (*Ovibos moschatus*) in Greenland and abortions and neonatal deaths have been recorded in certain captive antelopes (*Boselaphus tragocamelus*) (Kutz et al., 2000). Acute toxoplasmosis has been found in captive gazelles (*Gazella cuvieri, G. leptoceros, Nanger dama, Litocranius walleri*) in North American zoos (Dubey et al., 2004). Toxoplasmosis has been also identified in zoo birds like canaries and the black-winged lory (*Eos cyanogenia*) (Weiss and Kim, 2007). Congenital toxoplasmosis has been found in lynx cubs (*Lynx rufus*). Lynxes are considered important in perpetuating toxoplasmosis in the population of herbivores in the US (Borkovcova, 2003; Ryser-Degiorgis et al., 2006). In Victoria, British Columbia, oocysts excreted by pumas (*Puma concolor*) and spread in water are believed to be responsible for certain outbreaks of humans toxoplasmosis (Aramini et al., 1998). Experimental infections resulting in the excretion of oocysts through feces have been performed in jaguars (*Puma yagouaroundi*), ocelots (*Felis pardalis*), lynxes (*Lynx rufus*) and cheetahs (*Acinonyx jubatus*) (Mucker et al., 2006). However, the role of these felines in the excretion of oocysts is less significant than that of the domestic cat (Weiss and Kim, 2007).

Clinical toxoplasmosis has not been found in bears. Viable forms of *T. gondii* have been detected in the American black bear (*Ursus americanus*) and serological testing has determined the presence of the parasite in polar bears (*Ursus maritimus*) and grizzly bears (*Ursus arctos*) (Zarnke et al., 1997; Rah et al., 2005). Therefore, bear meat can be considered
a source of infection for carnivores (Weiss and Kim, 2007). Tests performed on raccoons (*Procyon lotor*) indicate a high prevalence of the infection in this host (Hancock et al., 2005). There are some scarce reports on cystic parasitic formations in raccoon tissue, but attempts to identify the disease by immunohistochemistry have failed (Weiss and Kim, 2007).

A fatal form of toxoplasmosis has been identified in three rabbits (*Oryctolagus cuniculus*) in two different areas in the US (Figueroa-Castillo et al., 2006). The rabbits died of an acute form of the disease. Similar forms of the disease have been found in rabbits aged 2 to 18 months in 15 farms in Germany (Weiss and Kim, 2007). The presence of *T. gondii* has been signalled in Cervidae in North America. Consumption of deer meat has been linked to the clinical form of the disease in humans. Naturally infected deer have not shown any clinical symptoms, but the parasite has been isolated in tissues collected from white-tailed deer (*Odocoileus virginianus*), and in mule deer (*O. hemionus*) (Dubey et al., 2009). In the reindeer (*Rangifer tarandus*), congenital toxoplasmosis has been identified and in such cases the disease was shown to lead to enteritis and even death, after an experimental oral inoculation with oocysts. *Toxoplasma gondii* has been found in roe deer as well. The prevalence of *T. gondii* infection in Cervidae varies between 55.55% and 64.2% (Dubey et al., 2008; Gamarra et al., 2008).

**References**


Dărăbuş G., Oprescu I., Morariu S., Mederle N. 2006. [Parasitology and parasitic diseases] [in Romanian]. Editura Mirton, Timişoara.


