Screening for toxoplasmosis on wild animals in captivity using serology and molecular analysis

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Abstract. Serum samples (n. 11) from wild mammals kept in captivity were analysed by a multi species ELISA. Faecal samples (n. 102) from wild felids kept in captivity were processed for DNA extractions and subsequent nested PCR analysis. These animals live in contact with humans and are often fed with raw meat. The results on the serum samples showed that all zoo samples were positive while the samples from a single circus were negative. The molecular results on faecal samples are all negative, but oocysts being shed through faeces in cases of acute infection in the felids last for only few days in their entire life.

Keywords: toxoplasmosis, *Toxoplasma gondii*, wild animals, serology analysis, molecular analysis.

INTRODUCTION

Toxoplasma gondii is a coccidian protozoa that can infect different tissues in warm-blooded animals. The seroprevalence ranges from 20 to 80 % and is quite spread worldwide in humans and animals. The life cycle of the parasite includes a sexual stage that can occur only in the gut of felids (definitive host) and an asexual stage that can occur in many tissues in various warm-blooded animals from birds to mammals (intermediate host) (Dubey, 1977). Epidemiological data indicate that exposure to oocysts (contaminated soil, water and vegetables) and undercooked meat are the primary risk factors for human infection (Jones et al., 2001). The parasite is also spread in free-living wild animals (Fredebaugh et al., 2011). A screening of wild animals kept in captivity in zoos and in different circuses has been started to estimate the exposure risk of toxoplasmosis in urban environment. Wild animals in captivity may pose a risk to the experts and operators.

MATERIALS AND METHODS

Data reported in this paper are from animals referred by Italian Zoos and circuses from throughout the country.

Sampling

Frozen sera (11 sampling) were collected in a period from 2005 to 2012. In 2011 tissue samples from three dead animals (a lion, a panther and a squirrel monkey) were also analysed. Faecal samples were collected in 2011 from the wild felids (Fig. 1). Almost fifty grams of faeces from different areas of the cage/s in which animal/s is/are maintained were collected and pooled to reach up to 500 grams. Faecal samples were frozen and sent periodically to Centro di Referenza Nazionale per la Toxoplasmosi (Ce. Tox.) at the Istituto Zooprofilattico Sperimentale della Sicilia, Palermo (Southern Italy).

Serology analysis

Sera were analysed for detection of antibodies against *T. gondii* by using an indirect multi-specie ELISA diagnostic kit (ID.VET, code TOXOS 0907).

Molecular analysis

The DNA was extracted from faecal and tissues samples using the QIAamp DNA stool kit (Quiagen cat n. 51504) and the PureLink Genomic DNA kit (Invitrogen k1820-02), respectively. To exclude the presence of Taq DNA polymerase inhibitors in the DNA extracted from the stool 1/20 sample was analysed in duplicate by adding *T. gondii* as a positive control.

The PCR analysis was performed by using the following primers:

NC 18S forward *tgcggaaggatcattcacacg*	NC 28S reverse *ccgttactaagggaatcatagtt*
first PCR (Vitale et al., 2008)	
ITS1 forward *gatttgcattcaagaagcgtgatagtat*	ITS1 reverse *agtttaggaagcaatctgaaagcacatc*
nested PCR (Jaregui et al., 2001)	

Fig. 1 – Fecal sampling in a *Panthera tigris*.

Tab. 1 – Analysis on sera from different animals collected from the year 2005 to date.

Serum samples from Zoo 1 and Zoo 2 **All samples resulted positive**		**Serum samples from from Circus I** **All samples resulted negative**		
Animal species	*Date of sample collection*	*Animal species and name*	*Date of birth*	*Date of sample collection*
Leopard *(Panthera pardus)*	01/06/2007	Tiger *(Panthera tigris)* Jessica	25/06/09	7/2/2012
Tiger *(Panthera tigris)*	08/06/2007	Tiger *(Panthera tigris)* Mala	25/06/09	7/2/2012
Tiger *(Panthera tigris)*	10/07/2008	Tiger *(Panthera tigris)* Rumba	25/06/09	7/2/2012
Asian black bear *(Ursus thibetanus)*	28/11/2005	Tiger *(Panthera tigris)* Samba	23/04/04	7/2/2012
Asian black bear *(Ursus thibetanus)*	09/06/2007	Tiger *(Panthera tigris)* Elsa	23/04/04	7/2/2012
Lion *(Panthera leo)*	16/11/2010			

Tab. 2 – Felid species and number of the collected faecal samples analysed.

Zoo 3 **Northern Italy**	**Zoo 4** **Northern Italy**	**Zoo 5** **Central Italy**	**Circus II**	**Circus III**	**Circus IV**	**Circus V**
1 European wild cat (*Felis silvestris*)	5 Leopards (*Panthera pardus*)	1 Leopard (*Panthera pardus*)				
20 Tigers (*Panthera tigris*)	8 Tigers (*Panthera tigris*)	3 Tigers (*Panthera tigris*)	10 Tigers (*Panthera tigris*)	7 Tigers (*Panthera tigris*)	15 Tigers (*Panthera tigris*)	13 Tigers (*Panthera tigris*)
5 Lions (*Panthera leo*)	5 Lions (*Panthera leo*)	3 Lions (*Panthera leo*)			13 Lions (*Panthera leo*)	
1 Siberian tiger (*Panthera tigris altaica*)	2 Pumas (*Puma concolor*)	3 Pumas (*Puma concolor*)				

RESULTS

Serology analysis

The results on the serum samples are reported in table 1.

The results showed that all zoo samples were positive while the samples from a single circus were negative.

Molecular analysis

A total of 102 faecal samples were tested by PCR. The analysis was performed on faecal samples from single animal or from group of animals. In some cases collection were repeated at two different seasons. All faecal samples tested negative (table 2).

This result should however take in to account that oocysts are shed through faeces in cases of acute infection in the felids and last for only few days in their entire life. False negative results cannot be excluded due to the presence of Taq DNA polymerase inhibitors in the stool even though in our studies, for every 20 analyses, one sample was spiked with *T. Gondi* DNA as a control and did show a positive result. Although the oocysts can persist in the environ-

ment for longer periods, their dispersion in urban setting can be facilitated by the presence of many passive carriers in a narrow space (birds, insects, mice, lizards, dogs) and from human activities (washing of the cages, frequent transfer of the animals etc.). Tissues collected from deceased animals also showed negative results by PCR.

DISCUSSION

Further analysis is necessary for a better estimation of *T. gondii* seroprevalence in captive animals, living in close contact with the humans and their pets, especially dogs that are mechanical carriers of *T. gondii* (Frenkel et al., 2003). Furthermore, the animals of zoo and circus are often fed meals derived from the same animal carcasses, vegetables and fruits that are directed to the human consumers' tables as it occurs for milions of pets and livestock that have more close contact with humans and their dwelling. The animals that lived in circuses were fed essentially with discarded broiler chicken meat, which is probably *Toxoplasma* free considering the chicken age and the broilers breeding conditions. Toxoplasmosis screening in these animals may give an estimate of the parasitic load in food intended for human consumption.

RIASSUNTO

Studi molecolari e sierologici sulla toxoplasmosi negli animali selvatici tenuti in cattività

Sono stati raccolti campioni fecali da felidi selvatici e campioni sierologici da animali selvatici a sangue caldo, tenuti in circhi o zoo, con l'intento di eseguire uno studio epidemiologico sulla toxoplasmosi negli animali selvatici mantenuti in cattività. I campioni sierologici sono stati analizzati attraverso un ELISA generico per tutte le specie. I campioni fecali sono stati processati per estrarre il DNA quindi sono stati analizzati per mezzo di una nested PCR. I campioni fecali sono risultati tutti negativi mentre diversi campioni sierologici sono risultati positivi. Questi animali vivono in stretto contatto con l'uomo e sono tenuti nei pressi di centri urbani. Sono spesso alimentati con scarti di frutta e verdura provenienti da fornitori alimentari locali e con carni provenienti dalla macellazioni di animali destinati al consumo umano. Questo studio preliminare può rappresentare un utile mezzo per stimare la carica parassitaria di *Toxoplasma gondii* sul territorio in relazione alla presenza di animali selvatici in stretto contatto con l'uomo e l'ambiente urbano oltre che valutare l'esposizione al rischio di infestazione nei centri urbani.

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