First record of Toxoplasma gondii *antibodies in Royal Bengal tigers (*Panthera tigris tigris*) and Asiatic lions (*Panthera leo persica*) in India*

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Keywords
Biochemical parameters,
Haematological parameters,
Indirect ELISA,
India,
Toxoplasmosis,
Wild felids,
Zoological park.

Summary
The purpose of this study was to detect the antibodies against *Toxoplasma gondii* in Royal Bengal tigers (*Panthera tigris tigris*), Asiatic lions (*Panthera leo persica*), leopards (*Panthera pardus*), and elephants (*Elephas maximus indicus*) residing in the Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab (India) during winter and monsoon seasons. Using indirect ELISA, 20 serum samples were analysed during the winter season. Results indicated that 1 lion (5%) tested seropositive, and 3 tigers and 1 lion (20%) were considered suspect. During the monsoon, 4 individuals (2 tigers and 2 lions, 20%) were seropositive, whereas only 1 tiger (5%) gave suspected results. Significantly higher globulin, creatinine, blood urea nitrogen, phosphorus, and creatine kinase values were recorded in seropositive and suspected groups. Levels of albumin, glucose, calcium, sodium, and iron decreased significantly in the seronegative group. Results from sero-testing 40 rodents trapped in and around the park depicted the presence of antibodies against *Toxoplasma gondii* in 1 individual. This study reveals the haemato-biochemical alterations in both seropositive and suspected wild felids for toxoplasmosis. Moreover, it provides the first serological evidence of *T. gondii* exposure in wild felids, notably Royal Bengal tigers and Asiatic lions, in India.

*Presenza di anticorpi anti-*Toxoplasma gondii *in tigri reali del Bengala (*Panthera tigris tigris*) e leoni asiatici (*Panthera leo persica*) in India*

Parole chiave
India,
Parametri biochimici,
Parametri ematologici,
ELISA indiretto,
Toxoplasmosi,
Felini selvatici,
Parco zoologico.

Riassunto
Scopo di questo studio è stato quello di indagare sulla presenza di anticorpi anti-*Toxoplasma gondii* in tigri reali del Bengala (*Panthera tigris tigris*), leoni asiatici (*Panthera leo persica*), leopardi (*Panthera pardus*) ed elefanti (*Elephas maximus indicus*) del Mahendra Chaudhury Zoological Park, Chhatbir, Punjab (India). Su 20 campioni di siero prelevati durante la stagione invernale ed esaminati in ELISA, 1 campione di siero prelevato da un leone (5%) è risultato positivo mentre 3 prelevati da tigri e uno da un altro leone (20%) hanno dato esito dubbio o sospetto; nel prelievo effettuato dagli stessi animali nel periodo dei monsoni, 4 campioni di siero, prelevati da 2 tigri e 2 leoni (20%), sono risultati positivi mentre un campione prelevato da una terza tigre (5%) ha dato esito dubbio o sospetto. Rispetto ai negativi, gli animali risultati sieropositivi e dubbi o sospetti all'ELISA hanno evidenziato valori di globulina, creatinina, azoto ureico, fosforo e creatina chinasi significativamente più elevati. Analogamente, i livelli di albumina, glucosio, calcio, sodio e ferro rilevati nel gruppo sieronegativo sono risultati significativamente più bassi. La presenza di anticorpi anti-*Toxoplasma gondii* è stato rilevata anche in un roditore catturato nelle aree adiacenti al parco. Questo studio evidenzia per la prima volta l'esposizione di tigri reali del Bengala e leoni asiatici al *T. gondii*. Dimostra inoltre un'associazione significativa tra sieropositività e alterazione di alcuni parametri ematici e biochimici.

Introduction

Toxoplasma gondii, an obligate intracellular protozoan parasite, is the etiological agent of the ubiquitous zoonotic disease, toxoplasmosis (Tedesco 2004). It has the potential to infect almost all homeothermic animals (Lopes *et al.* 2008, Liu *et al.* 2012). Approximately, one-third of the world human population is chronically infected with *T. gondii* (Tenter *et al.* 2000). Toxoplasmosis can be harmful in immunocompromised humans and pregnant women, especially if a woman is infected during the first trimester of her pregnancy (Dubey and Beattie 1988). It is also responsible for causing abortions in sheep (Dubey and Beattie 1988).

Domestic and wild felids are the only definitive hosts for *Toxoplasma gondii*. They thus play the most important role in the life cycle of the detrimental parasite, as they shed environmentally resistant infective oocysts (Miro *et al.* 2004). The oocysts shed by infected wild felids in captivity are not only a source of infection to other non-infected captive wild felids, but also to other zoo animals, zoo keepers, zoo veterinarians, as well as to the persons visiting zoos (Thiangtum *et al.* 2006).

In India, conservation strategies for the Royal Bengal (RB) tigers (*Panthera tigris tigris*) were recently implemented following a serious reduction in the RB population (Banerjee 2013). Though toxoplasmosis is asymptomatic in felids and rarely results in clinical signs, it can result in breeding failure in felids (Thiangtum *et al.* 2006). Serological studies are better suited to detect *T. gondii* infection in comparison to faecal sample examination for oocysts because wild felids shed the oocysts for only a shorter period of time (Dubey and Thulliez 1989).

The significant role of *T. gondii* in the pathophysiology of wild felids and the epidemiological role of wild felids in the transmission of toxoplasmosis in India has not yet been established. This study was planned to adjudge the seropositivity of *T. gondii* in RB tigers, Asiatic lions (*Panthera leo persica*), leopards (*Panthera pardus*), and elephants (*Elephas maximas indicus*), and thus address the lack of relevant literature regarding the prevalence of toxoplasmosis in wild felids in this region. It also aims to compare the associated alterations in the haemato-biochemical parameters of the seropositive individuals to those of healthy seronegative animals.

Materials and methods

Study area and management conditions

The Punjab state is located between 29" 30' N to 32" 32' N latitude and 73" 55' E to 76" 50' E longitudes at an altitude ranging between 180 to 300 metres above main sea level. The zoological park (Mahendra Chaudhury Zoological Park, which falls under sub-mountain undulating zone of the province) considered in this study is comprised of animals and birds belonging to around 61 different species, some of which were rare or endangered. The animals targeted in this study were kept in close confinements with adequate wandering space. The wild felines were fed with buffalo meat (Carabeef) and *ad libitum* water; elephants were provided with green fodder and sugarcane with *ad libitum* water.

Ethical aspects and sampling frame

The ethics committee for animal experiments of Guru Angad Dev Veterinary and Animal Sciences University granted the ethical approval (IAEC/2013/27-43) to conduct this study. The blood samples from 11 RB tigers, 3 Asiatic lions, 2 leopards, and 4 elephants (negative control) were collected twice (in February and August – across winter, monsoon, and summer seasons) in 2013. The wild animals were restrained in squeeze cages and blood samples were taken from the dorsal coccygeal vein. The blood samples were collected in vacutainers with anticoagulant and were stored at - 20°C directly for haematological parameters. Sera were separated from the blood samples collected in the vacutainers with coagulation activators by centrifugation and stored at - 20°C for further immunological and biochemical analysis.

Serological screening

A commercial indirect ELISA (iELISA) kit from Cusabio® (Wuhan Hi-tech Medical Devices Park, China) was used in order to detect *T. gondii* antibodies. The kit was used according to the manufacturer's instructions. The percent positivity (%P) of samples was obtained as follows:

$$\text{Percent positivity (\%P)} = \frac{\text{Mean OD of Sample}}{\text{Mean OD of Critical control}} \text{ X100}$$

OD is the optical density of the sample. This is compared with the optical density of the critical control (provided in the kit), and helps in evaluating the values of percent positivity of the samples.

Samples with %P value less than 90% were considered seronegative. Those with more than 110% were considered seropositive, and the values in-between were considered suspected. Thus, the animals were divided into 3 groups, as per the infectivity pattern: seropositive, suspected, and seronegative, and further analyses of haemato-biochemical alterations were carried out among these groups.

Sero-testing of wild rodents (n = 40) was also

carried out in order to assess their probable role in the transmission of toxoplasmosis to wild felids. The rodents were captured from the fields and jungle area around the zoological park by applying rat-catcher machines, and blood was collected by using the retro-orbital plexus puncture method.

Haemato-biochemical analysis

The haematological analysis of the blood samples for all of the animals (except leopards and elephants) was carried out on fully automated analyser, ADVIA 2120 Haematology system (Siemens Health Care Diagnostic Inc. Deerfield, IL, US). Analysis included haemoglobin level (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV), and differential leukocyte count (DLC). Biochemical parameters were analysed by using commercial kits of Siemens Health Care Diagnostics Inc. IL, U.S.A. These included total bilirubin (TBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), total protein (TP), albumin (ALB), globulin (GLO), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), creatine kinase (CK), blood glucose (GLU), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (P), and iron (Fe). Thus, haemato-biochemical alterations were studied in detail only for RB tigers and Asiatic lions, as the levels of all the haematological and biochemical parameters fell within normal range in the cases of leopards and elephants.

Statistical analysis

The analysis of variance (one-way ANOVA) of haemato-biochemical parameters among the different groups was done using SPSS 16.0 software (Marco *et al.* 2000).

Results

At the beginning of the study period (winter), 1 animal was seropositive (lion) and 4 were suspected (3 tigers and 1 lion) for *T. gondii* by iELISA. By the end of the monsoon season, 4 animals were seropositive (2 tigers and 2 lions) and 1 (tiger) was suspected. The antibody titres of 2 tigers and 1 lion exceeded the positive threshold value by the end of the study period. The anti-*Toxoplasma* antibody titres showed only a slight increase with the change of seasons, while the animals in seronegative titre range at the beginning of the study remained in the same range. All the elephants and leopards were seronegative in both seasons (Figure 1), with percent optical density (OD) values ranging between 57.54%-78.82% and 30.53%-57.07% for leopards and elephants, respectively.

The serum biochemical studies targeting seropositive,

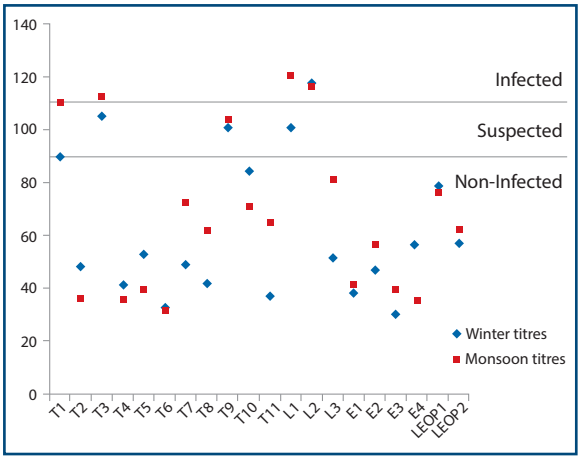

Figure 1. *Antibody titres against* Toxoplasma gondii *in Royal Bengal tigers (T), Asiatic lions (L), Indian elephants (E), leopards (LEOP) in Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India.*

Table I. *Serum biochemical values in captive Royal Bengal tigers and Asiatic lions of the Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India according to* Toxoplasma *infectious status.*

Biochemical Parameters / Groups	TBIL (mg/dL)	AST (U/L)	ALT (U/L)	ALKP (U/L)	TP (g/dL)	ALB (g/dL)	GLO (g/dL)	BUN (mg/dL)	CRE (mg/dL)	GLU (mg/dL)
Group I (Seropositive)	0.74±0.50*	41±14.25*	51.2±7.79*	33.4±6.91*	7.56±0.53*	3.26±0.26*	4.3±0.43*	69.6±11.95*	3.26±0.50*	29.8±7.01*
Group II (Suspected)	0.66±0.34*	45.4±18.90*	46.4±10.55*	32.8±4.21*	7.38±0.19*	3.26±0.24*	4.12±0.18*	64.2±8.67*	3.06±0.39*	23±3.74*
Group III (Seronegative animals)	0.96±0.38*	51.5±10.13*	46.11±10.81*	33.28±6.73*	7.36±0.46*	4.03±0.44**	3.33±0.36**	50.61±5.19**	2.08±0.29**	68.72±12.5**
Normal range (NR) Shrivastav *et al.* 2011 (Tigers), Jani and Sabapara 2010 (Lions)	0.4-3.2	14.4-84.0	21.2-109.0	16.28-87.9	3.7-8.7	2.1-4.6	1.6-4.1	6.5-48.2	1.6-4.6	66-124

Values indicated as Mean± Standard deviation; *Values differing significantly at $P < 0.05$;
TBIL = Total bilirubin; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALKP = Alkaline phosphatase; TP = Total protein; ALB = Albumin; GLO = Globulin; BUN = Blood urea nitrogen; CRE = Creatinine; GLU = Glucose.

suspected, and seronegative groups including tigers and lions revealed no significant difference in the values of TBIL, AST, ALT, ALKP, and TP and values of all the parameters were in the normal range in both seasons irrespective of the group (Table I). The values of ALB recorded in the seropositive and suspected group were significantly ($P < 0.05$) lower than the values recorded in the seronegative group (Table I). The globulin level showed a significant increase in the seropositive and suspected groups as compared to the seronegative group.

The BUN values in both seasons for both seropositive and suspected groups were significantly ($P < 0.05$) higher than those recorded in the seronegative group (Table I). The creatinine levels of the animals in the seropositive and suspected groups were significantly higher than the animals of the seronegative group. The glucose levels of the seropositive and suspected groups were significantly ($P < 0.05$) lower than those recorded in animals of the seronegative group. Individually, the glucose concentration values for all of the suspected and seropositive tigers and lions were below the normal range, whereas the values for seronegative individuals were within the normal range (Table I).

The values for UA, K, and Cl did not vary significantly among any of the 3 groups. The values of Ca, Na, and Fe in the suspected and seropositive groups were significantly ($P < 0.05$) lower than the seronegative group, whereas the levels of P and CK were significantly higher in the animals of the seropositive and suspected groups than the values recorded for seronegative animals (Figures 2 and 3).

All the haematological parameters varied non-significantly except for TLC and neutrophils percentage, where the values recorded in the seropositive and suspected groups were significantly

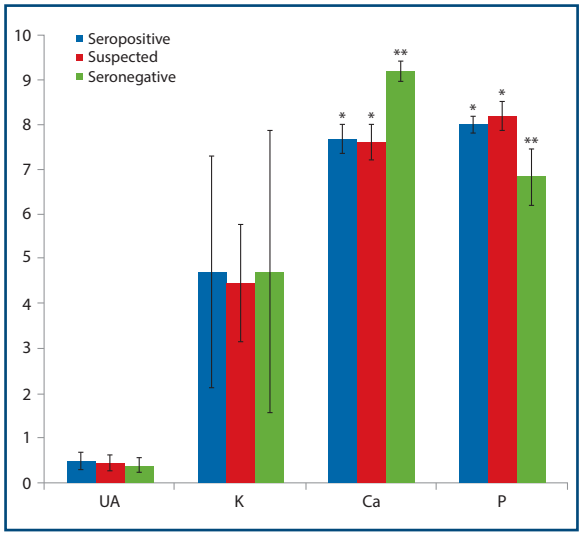

Figure 2. *Uric acid (UA), potassium (K), calcium (CA) and phosphorus (P) values in captive Royal Bengal tigers and Asiatic lions of the Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India according to Toxoplasma infectious status.* Superscripts '*' and '**' indicate values differing significantly ($P < 0.05$).

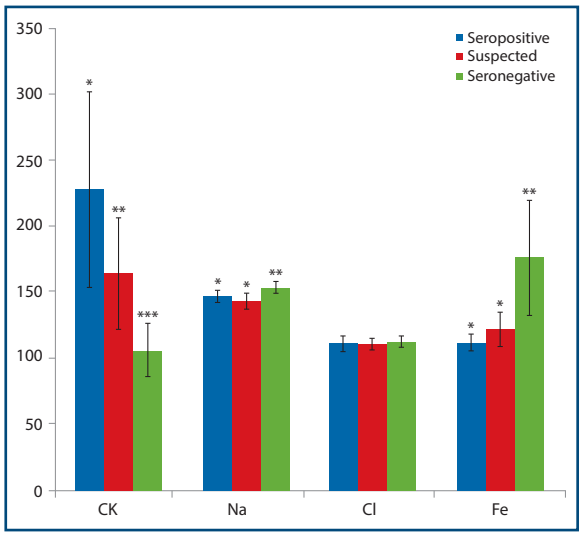

Figure 3. *Creatinine kinase (CK), sodium (Na), chloride (Cl) and iron (Fe) values in captive Royal Bengal tigers and Asiatic lions of the Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India according to Toxoplasma infectious status.* Superscripts '*', '**' and '***' indicate values differing significantly ($P < 0.05$).

Table II. *Haematological values in captive Royal Bengal tigers and Asiatic lions of the Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India according to* Toxoplasma *infectious status*

Haematological Parameters / Groups	Hb	TLC	TEC	PCV	Platelets	N	L	E	M
Group I (Seropositive)	14.02±1.39*	18,840±2,013.34*	8.53±0.69*	41.08±4.86*	204.2±25.53*	76.2±7.22*	20.6±5.55*	2±2.34*	0.8±1.79*
Group II (Suspected)	13.04±2.46*	17,652±1,964.72*	8.46±1.26*	38.64±6.11*	274±46.78*	76.4±9.20*	22.4±9.21*	1.2±1.79*	0*
Group III (Seronegative)	14.72±1.03*	14,418±1,538.89**	8.14±0.70*	42.98±7.62*	220.78±51.34*	68.94±2.62**	29.28±2.99*	1.11±1.41*	0.5±1.15*
Shrivastav *et al*. 2011	7.8-13.8	6,200-11,050	4.66-9.15	36-45	-	57-75	18-35	2-6	2-6

Values are indicated as Mean± Standard deviation; *Values differing significantly ($P < 0.05$);
Hb = Haemoglobin level; TEC = Total erythrocyte count; TLC = Total leukocyte count; PCV = packed cell volume; Platelets = platelets and components of differential leukocyte count *i.e.* N = neutrophils; L = lymphocytes; E = eosinophils; M = monocytes.

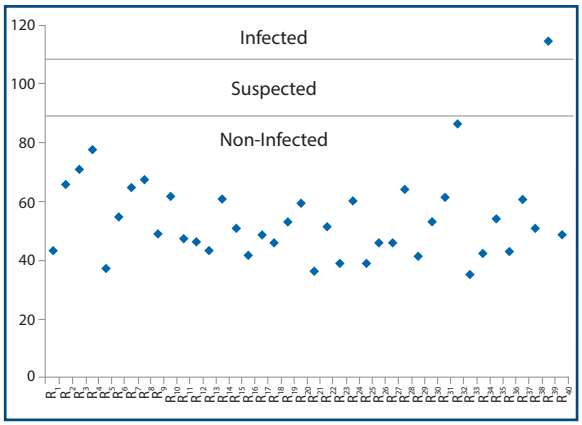

Figure 4. *Antibody titres against* Toxoplasma gondii *in wild rodents (R_1-R_{40}) captured in and around Mahendra Chaudhury Zoological Park, in Chhatbir, Punjab, India.*

($P < 0.05$) higher than the values recorded in the seronegative group (Table II). The TLC values for all the groups were higher than the normal range.

Only 1 serum sample of wild rodent was seropositive for *T. gondii* (Figure 4).

Discussion

This study highlights the presence of antibodies against *T. gondii* infections in captive wild felids (RB tigers and Asiatic lions) living in a zoological garden. Though the sero-detection of toxoplasmosis has been carried out in domestic ruminants in the past (Selvaraj *et al.* 2007, Sharma *et al.* 2008), there has never been a study that considers wild felids. This investigation recorded the first serological evidence of *T. gondii* exposure in RB tigers and Asiatic lions in India, and moreover found that seropositivity increased from winter to monsoon. This may be due to the fact that parasites were in incubation stage during the winter with no/fewer detectable anti-*T. gondii* antibodies. These levels increased during the monsoon season. This increase in the number of seropositive individuals could be attributed to stressful conditions (environmental stress caused by heat and humidity) encountered by the animals during the summer and monsoon seasons, which would result in the immunosuppression of the animals that would, in turn, cause such an increase (Salant and Spira 2004). Another factor could be the increase in moisture during the monsoon season. The survival of oocysts is prolonged in cool, moist conditions, which in turn would aggravate the infection in these animals (Bisson *et al.* 2000). The management of conditions in the zoological gardens therefore has a direct impact on the susceptibility of animals to contract infections.

The most frequent cause of toxoplasmosis infection in definitive hosts is through the ingestion of tissues from an infected intermediate hosts (Dubey and Beattie 1988, Thiangtum *et al.* 2006). Thus, infected raw buffalo or chicken meat offered to captive wild felids could be responsible for spreading infection. Anti-*Toxoplasma* antibodies were detected in the serum sample of 1 rodent. Their possible role in the transmission of the parasite to the captive felids could therefore not be neglected. Other routes of transmission might have involved mechanical transmission by flies, cockroaches, or dung beetles entering into the living area of the wild felids (Thiangtum *et al.* 2006). The elephants and leopards displayed negative antibody titres and the comparatively lower titre values of the elephants could be attributed to their herbivorous nature. The values of the serum biochemical parameters (ALT, AST, TBIL, and ALKP) were within the normal range, which is consistent with the findings of Mosallanejad and colleagues (Mosallanejad *et al.* 2007). The increased levels of the globulins in both seropositive and suspected groups could be attributed to the presence of immunoglobulins generated against an ongoing infection (Sedlack and Bartova 2006). The higher creatinine levels that were recorded were suggestive of *T. gondii* infection in the definitive hosts (Lappin 1996). Toxoplasmosis is usually asymptomatic in cats, however the decreased blood glucose levels that were observed in this study can be correlated with the lethargy observed in *T. gondii* seropositive felids (Elmore *et al.* 2010). The significantly increased creatine kinase levels in seropositive and suspected animals influenced the brain or muscles in animals belonging to these two groups and resulted in lowered response times. The decreased serum Ca levels revealed the involvement of the pancreas in chronic toxoplasmosis – a condition that leads to pancreatitis in definitive hosts (Advincula *et al.* 2010).

The neutrophilic leucocytosis observed in this study is consistent with the findings of Mosallanejad and colleagues (Mosallanejad *et al.* 2007), who considered it as a major finding relating to toxoplasmosis. Other important attributes of the *T. gondii* infection, including anaemia and jaundice, were not observed in this study. This may be due to the chronic nature of the infection in these animals (Advincula *et al.* 2010).

This study is the first report of captive wild felids (of India) exposure to *T. gondii*. It indicates that captivity (in zoological gardens) fosters stress and further immunosuppression and renders the definitive hosts (wild felids) vulnerable to toxoplasmosis. The haemato-biochemical alterations may prove good indicators for the adverse aftermaths of this intestinal protozoan parasite while undergoing extra-intestinal life cycle. Wild felines seropositive for *T. gondii* risk infecting other animals in the zoological garden as well as the individuals (zoo keepers and veterinarians) associated with the management of

animals. Further strategies should be implemented to manage this infection.

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