Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

Toxoplasma gondii infection in European mouflons (*Ovis musimon*) and captive wild felines from Puebla, México

Luis Fernando Valenzuela-Moreno [a], Carlos Cedillo-Peláez [a], Claudia Patricia Rico-Torres [a], María Alejandra Hernández-Rodríguez [b], María del Carmen Carmona-Muciño [b], José Eduardo Farfán-Morales [c], Dolores Correa [a], Heriberto Caballero-Ortega [a,*]

[a] *Laboratorio de Inmunología Experimental, Instituto Nacional de Pediatría, Insurgentes Sur 3700-C, Colonia Insurgentes-Cuicuilco, Delegación Coyoacán, C.P, 04530, Ciudad de México, Mexico*
[b] *Colección Zoológica Privada, Puebla, Pue, Mexico*
[c] *Departamento de Anatomía Patológica, Instituto Nacional de Pediatría, Insurgentes Sur 3700-C, Colonia Insurgentes-Cuicuilco, Delegación Coyoacán, C.P, 04530, Ciudad de México, Mexico*

ARTICLE INFO

ABSTRACT

The presence of *Toxoplasma gondii* in zoos is cause of alert because many susceptible species kept in captivity die of clinical toxoplasmosis. Moreover, excretion of *T. gondii* oocysts by infected captive wild felines into the facilities could pose a risk to workers. Herbivores in wild collections can serve as sentinels of local transmission, since they get infected by the consumption of oocysts present in ground or water. Both herbivores and felids may reveal the parasite variants which are circulating in the region. We determined the seroprevalence of *T. gondii* in European mouflons (n = 55) and wild felines (n = 15) from a private zoological collection located in the Eastern region of México, as well as the incidence in 41 of the mouflons using ELISA. The prevalence of *T. gondii* in mouflons was 14.5% (n = 55) and 17.1% (n = 41) in 2011 and 19.5% in 2012. The estimated incidence was 9.8%–12.2%. In wild felines the frequency was 80%. Four sero-positive animals (two mouflons and the two oldest African lions) were euthanized. Histopathology, conventional PCR (for *B1* and SeqRep529 loci) and molecular characterization were carried out. All euthanized animals were positive to *T. gondii* by PCR. We identified a triple infection (I + II + III) in the brain of a mouflon. In conclusion, a high infective pressure of *T. gondii* in the collection was found, supported by changes in its prevalence in European mouflons. A high prevalence of infection in wild felines was determined. At least four genotypes of *T. gondii* are present in herbivores and carnivores, and one mouflon had a mixed infection.

1. Introduction

Toxoplasma gondii is a cosmopolitan pathogen that infects almost any warm-blooded vertebrate, i.e., mammals and birds. The success of the parasite lies in its ability to reproduce both sexually and asexually among different hosts (Grigg and Sundar, 2009). Susceptible species kept in captivity such as Australian marsupials, New World monkeys, lemurs and meerkats, can die due to toxoplasmosis (de Camps et al., 2008). If a feline kept in captivity is infected, it represents an added risk to infect staff (de Camps et al., 2008; Alvarado-Esquivel et al., 2013). Ungulates in semi-confinement wild collections can serve as sentinels of environmental contamination; for instance, herbivores can become infected by the consumption of oocysts while grazing or drinking water.

Besides this, carnivores can be infected by the ingestion of raw meat containing *T. gondii* tissue cysts (Ferreira et al., 2019). There are few studies of *T. gondii* infection in private or public collections of wild animals in México (Espinosa-Avilés and Martínez-Morales, 2007; Alvarado-Esquivel et al., 2013), and none in the Eastern region of the Country, which has favorable climatic conditions for *T. gondii* and is adjacent to one of the most populated urban regions of México. The studied animals are in semi-confinement, which may offer a greater exposure to the parasite than traditional zoos. The present study was aimed to determine seroprevalence of *T. gondii* in European mouflons (*Ovis musimon*) and wild felids (*Panthera leo* and *P. tigris*) that live in semi-confinement in a private zoological collection. We also detected DNA of *T. gondii* in tissues samples of animals as well as determined genotypes present there. This

---
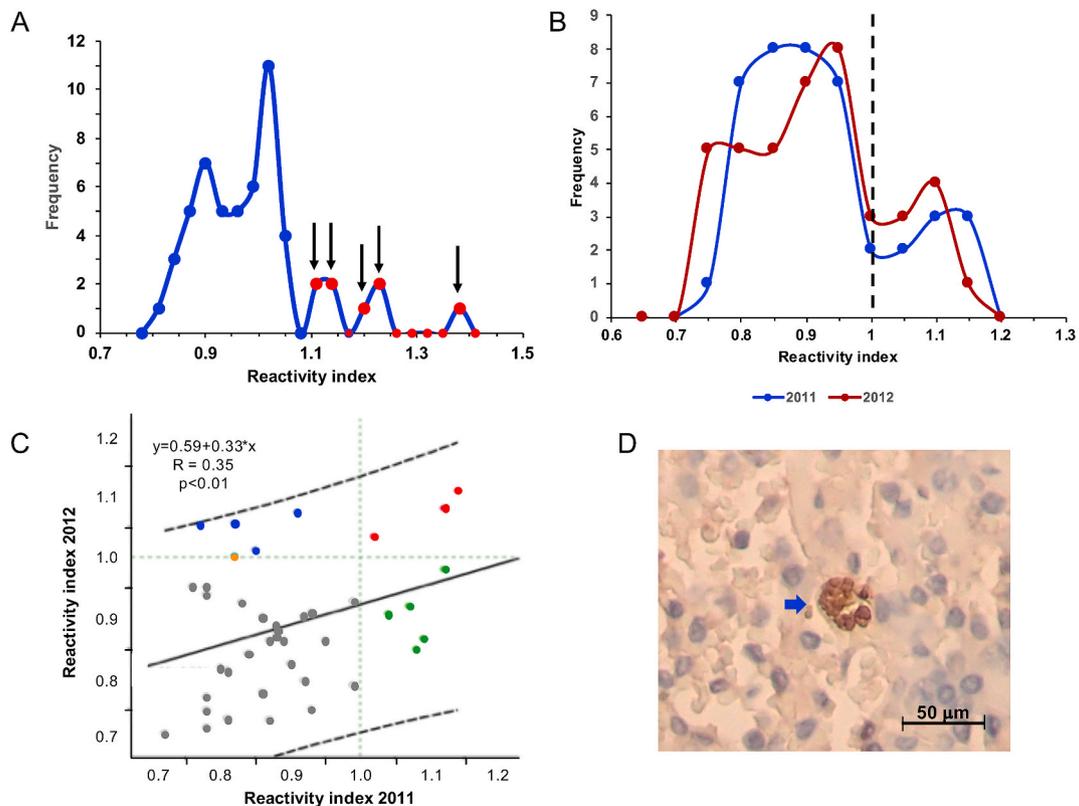
Fig. 1. *Toxoplasma gondii* infection in captive mouflons in 2011 and 2012. A. Anti-T. gondii frequency distribution in fifty-five sera of mouflons sampled in 2011; black arrows indicate positive samples as determined by their position to the right of the normally distributed values of the left population. B. One year later, 41/55 original mouflons were captured, bled, and re-tested for antibodies against this parasite. The cut-off point used was 1.0 RI (dotted lines), which separated the negative population (left) from the positive cases. C. Three mouflons remained positive, five became negative, and four seroconverted positive in 2012 (red, green and blue dots, respectively); one mouflon is on the cut-off (orange dot). D. Immunohistochemistry for *T. gondii* in tissues from in the spleen of mouflon 1, where an immunopositive cumulus of tachyzoites can be seen (blue arrow). The nuclei of resident lymphocytes and dendritic cells were contrasted with Meyer's hematoxylin. Bar: 50 μm. R = Pearson correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

is a cross-sectional prospective study and not from serum banks, as it has been commonly reported. In addition, we genotyped clinical samples collected specifically for this purpose.

2. Material and methods

2.1. Ethical approval

The present study was approved by the reviewing board of the Instituto Nacional de Pediatría of the Ministry of Health of México (INP; IRB-NIH numbers IRB00008064 and IRB00008065), which includes the Research and Animal Care Committees with registration number 013/2012.

2.2. Study site

The study was conducted in a private zoological collection localized at the municipality of Puebla, Puebla (18° 56′ 13.0″N, 98° 08′ 11.0″W), with an altitude of 2117 m above sea level, an annual temperature between 12 °C and 18 °C and annual rainfall from 700 to 1500 mm INEGI, (2020).

2.3. Collection of blood samples

In November 2011, 5 mL blood was collected from the jugular and saphenous veins from 55 randomly selected adult European mouflons (1–5 years old) and 15 wild felids [eight African lions (*Panthera leo*), and seven Bengal tigers (*P. tigris*)], respectively. The proportion of

individuals in the population that had anti-*T. gondii* antibodies at the time of sampling (prevalence) was determined. In November 2012, we found 41 of the 55 mouflons; they were sampled again and the incidence of *T. gondii* infection was determined (number of new cases of a disease in one year).

2.4. Serology

To detect specific antibodies against *T. gondii* in European mouflons and wild felids, we standardized an indirect ELISA using crude extract of the RH strain as antigen was performed as per Olamendi-Portugal et al. (2012), with some modifications; sera of mouflons and wild felids were diluted 1:400 and 1:200, respectively. Due to unavailability of a specific conjugate against mouflons, some secondary antibodies (anti-sheep, anti-goat, anti-bovine and protein G) were tested by direct ELISA using mouflon sera. The anti-goat conjugate was chosen because it recognized mouflon IgG with the highest sensitivity. Mouflon sera were tested with 1:10,000 dilution of the rabbit anti-goat IgG peroxidase conjugate (Sigma–Aldrich Corp., St Louis, MO, USA) (Supplementary figure). For sera of wild felids, the secondary antibody against cats coupled to peroxidase (Abcam, Cambridge, MA, USA, product ab112801) was used and diluted 1:15,000 (Valenzuela-Moreno, 2014). For mouflons and felines, three negative and three positive serum samples of naturally infected domestic goats and cats, respectively, were used as controls in each plate to determine the cut-off value, which was the mean plus 3 standard deviations of the values obtained with the three negative controls tested in duplicate, i.e., from six wells (Caballero-Ortega et al., 2008; Valenzuela-Moreno, 2014). Taking the absorbance of the positive

Table 1
Prevalence of anti-*Toxoplasma gondii* antibodies in captive mouflons and wild felines from Puebla, México.

Year	Species	Scientific name	Sex ratio (male: female)	No. positive/No. tested	Prevalence (%)	Incidence
2011	African lion	*Panthera leo*	4 : 4	6/8	75.0	–
	Bengal tiger	*Panthera tigris*	4 : 3	6/7	87.5	–
	European mouflon	*Ovis musimon*	16 : 39	8/55	14.5	–
			10 : 31	7/41	17.1	[a]9.8% (4/41) and 12.2% (5/41)
2012				8/41	19.5	

[a] One mouflon was on the cut-off point.

goat sera (direct positive control) as 100 percent, the absorbance obtained in each well (direct and experimental mouflon samples) was corrected. The modified absorbance value of each mouflon serum was divided by the cut-off point to establish the Reactivity Index (RI). For the detection of seropositive mouflons and wild felines, an RI equal to 1.0 was used as previously described (Caballero-Ortega et al., 2014). The RI frequency and the data correlation between 2011 and 2012 samples were plotted using frequency distribution curves and a scatter plot (Fig. 1).

2.5. Collection of tissue samples

Two mouflons with the highest RI of anti-*T. gondii* antibodies (M1 and M2) as well as the two aged (>24 years old) African lions (L1 and L2) included in the survey were euthanized with Sodium Pentobarbital (133 mg/kg body weight IV Doléthal®, Vétoquinol, France), according to the Mexican official standard NOM-033-ZOO-1995. Lions were previously anesthetized with a mixture of 2.0 mg/kg body weight Xylazine (Procin equus®, Pisa) plus 4.0 mg/kg body weight Ketamine (Ketamin-Pet®, Aranda) intramuscularly.

The necropsies were performed as per Schunemann and Constantino (2002). Tissue samples from heart, liver, spleen, brain and striated muscle (diaphragm and right *gracilis*) were collected. After the necropsy, one section of those tissues was fixed in 10% buffered formalin for histopathology, and other was frozen at −20 °C for DNA extraction, molecular detection and genotyping of the parasite.

2.6. Molecular assays

Genomic DNA from heart, liver, spleen, muscle and brain was obtained, using Qiagen Gentra® Puregene® Tissue kit, (Hilden, Germany), following the manufacturer instructions. For molecular diagnosis, DNA was amplified using previous described primers targeting *B1* gene and the non-coding 529 bp repetitive sequence (SeqRep529) (Pujol-Riqué, 1999; Homan and Vercammen, 2000). Multilocus nested PCR assays were performed to amplify 10 molecular markers (*SAG1, Alt. SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1* and *Apico*) following the methodology previously described; the amplified products were digested with specific enzymes (Su et al., 2010). The RH strain DNA and sterile water were included as positive and negative controls, respectively; in addition, positive and negative reamplification controls were also included in nested assays. For genotyping, DNA from RH, Me49 and VEG reference strains (type I, II and III respectively) were included. All PCR assays were carried out with Amplitaq Gold™ DNA polymerase (Thermo Fisher Scientific, cat. 4311806, MA, USA). In order to determine the parasite load in tissues, we followed the methodology previously described (Cedillo-Peláez et al., 2011), with modifications using primers and TaqMan® probe for SeqRep529 of *T. gondii* designed by Robert-Gangneux et al. (2010).

2.7. Histopathology and immunohistochemistry

Representative fixed tissue samples were stained with hematoxylin and eosin. For immunohistochemistry (IHC), a 1:250 dilution of *T. gondii*

positive goat serum (previously tested by ELISA and Western blot) was used as the primary antibody. A biotinylated mouse anti-goat IgG antibody (Biocare Medical, CAL, USA) diluted 1:300 was used as a secondary antibody. Immunocomplexes were detected with a commercial solution (Betazoid DAB, Biocare, Chromogen 3, 3′ Diaminobenzidine). Sections of liver or spleen of mice infected with tachyzoites of the Me49 strain were used as positive controls; as negative control, sections of the same tissue were used replacing the primary antibody with PBS (Cedillo-Peláez and MéxicoMéxico, 2015). Both HP and IHC slides were examined by optical microscopy (Zeiss Axiostar plus; Göttingen, Germany).

2.8. Statistical analyses

All data were analyzed using the SPSS v23.0 software (SPSS Inc., Chicago, IL, USA). The correlation between the sampling of 2011 and 2012, as well as between results of the first versus the second assays, was determined by R Pearson and Spearman correlation methods, with 95% confidence limits. Confirmation of the normal distribution of the lower absorbance values population was determined by Kolmogorov-Smirnov (K–S) test. In all cases a $p \leq 0.05$ was considered as statistically significant.

3. Results

In 2011, two populations of mouflons were identified in the frequency distribution of ELISA results: one grouped to the left of the histogram with normal distribution (K–S $p = 0.124$), while 8/55 (14.5%) were grouped to the right (Fig. 1A). One year later, 7/41 (17.1%) and 8/41 (19.5%) mouflons sampled in 2011 and 2012, respectively, were positives (Fig. 1B). From the 41 original mouflons re-tested one year later, three remained positive, five became negative and four seroconverted positive; one mouflon was on the cut-off (Fig. 1C, R = 0.35; p < 0.01). Therefore, the annual apparent incidence of *T. gondii* ranged between 9.8% (4/41) and 12.2% (5/41; considering one mouflon on cut-off as positive. Fig. 1C). Also, six out of eight lions (75%) and six out of seven tigers (85%) of the 15 wild felines analyzed were positive for anti-*T. gondii* antibodies, resulting in an overall prevalence of 80% (Table 1).

The two mouflons selected for euthanasia had good body condition (BCS 3/5) and overall good health, while the two felines were emaciated (BCS 1.5/5) and had chronic lesions associated with advanced age (AZA Nutrition Advisory Group, 2016; 2017). None of these animals had lesions compatible with those reported for acute or chronic infection of *T. gondii* during the necropsy or histopathology analyses. By IHC, immunopositive structures compatible with cysts or pseudocysts-like of *T. gondii* in the spleen and the muscle of mouflon 1 (M1) and brain of lion 2 (L2) were identified (Fig. 1D). We did not find *T. gondii* tissue cysts in the other two animals.

All euthanized animals (n = 4) were positive by conventional PCR of *B1* or SeqRep529 markers; in order to increase the chances to get enough DNA for genotyping, we chose those tissues that were positive for both loci to perform the multiplex nested PCR. A mixed infection was identified in the brain of M1 (I + II + III) at *SAG3* gene (Fig. 2; Table 2). The markers that could be genotyped were *SAG1,* Alt. *SAG2, SAG3* and *Apico*, which presented type I and III alleles. *BTUB, c22-8* and *PK1*
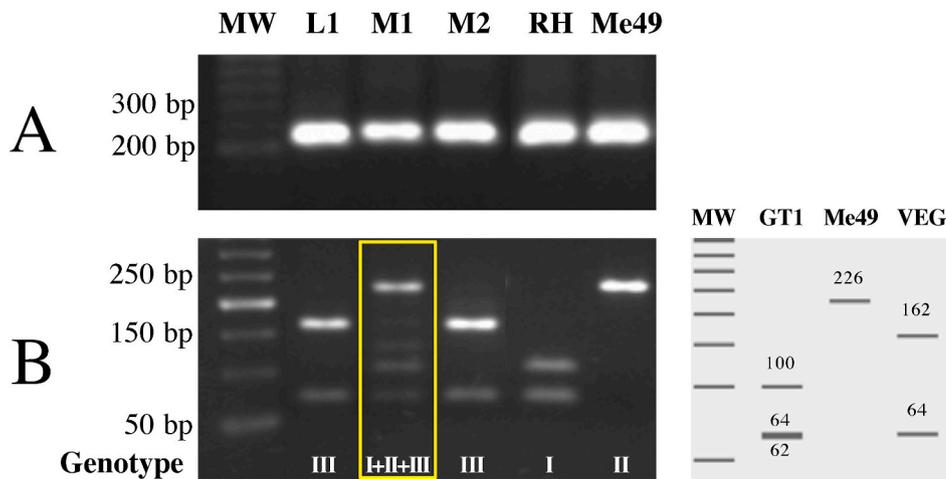
Fig. 2. Representative PCR-RFLP pattern of *T. gondii* for *SAG3* gene. Single and mixed infections in lions and mouflons tissues were observed. A. PCR for the *SAG3* locus of lion 1 (L1, spleen), mouflon 1 (M1, brain) and mouflon 2 (M2, liver) samples. B. A triple infection is highlighted (yellow box). Resulting genotypes are specified at the bottom. *In silico* digestion was done by www.benchling.com. Reference strains sequences GT1, TGGT1_308020; Me49, TGME49_308020; VEG, TGVEG_308020 are available at www.toxodb.org. MW: molecular weight marker; RH and ME49 are reference strains, type I and II, respectively; M: mouflon, L: lion. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

markers amplified the expected product, but due to the low amount of DNA they could not be genotyped. Parasite load could only be calculated in the samples of the mouflons, and values < 50 parasites per mg of tissue were obtained (Table 2).

4. Discussion

Prevention and control of toxoplasmosis is a significant issue in zoos and private collections, as wild felines and feral cats can excrete oocysts in their feces. These parasite forms could be disseminated through the zoo's facilities by rainfall water and mechanically by the zoo staff; by these means, captive species (some of them listed as endangered) may get infected and die due to toxoplasmosis (de Camps et al., 2008). In the present study we determined the exposure to *T. gondii* of herbivores and carnivores from a private zoological collection in the state of Puebla, México. The mouflons included in this study live in semi-confinement and therefore could have the possibility of being exposed to oocysts shed by feral and wild felines to water or grass that grows in the meadow. Previous studies showed higher prevalence of *T. gondii* in wild mouflons (15.0–23.0%) than in mouflons raised in total confinement (0.0–8.0%). We found high seroprevalence of *T. gondii* (14.5% or 17.1% in 2011 and 19.5% in 2012) as compared to that found in animals (0.0–8.0%) raised in traditional zoos (Hejlicek et al., 1997; Sedlák and Bártová, 2006; Aubert et al., 2010).

As far as we know, this is the first report of incidence of *T. gondii* in wild sheep (9.8%–12.2%), which was higher than that reported by Caballero-Ortega et al. (2008) in domestic ovines (2.1%) of an experimental farm in a tropical region between Puebla and Veracruz states of México. It must be highlighted that a few months later there was an outbreak of toxoplasmosis in this private collection, which caused the death of some susceptible individuals in the collection (unpublished data). This confirms that herbivores, by feeding on grass, are useful as sentinels of infection by *T. gondii* oocysts.

High seroprevalence reported in our study is concordant with other studies (Philippa et al., 2004; Yang et al., 2017; Ferreira et al., 2019). For instance, 81.3% of captive wild cats tested in three zoos of Mexico City were seropositive (Alvarado-Esquivel et al., 2013). The routine diet of the wild felids included chicken and horse meat, which may contain tissue cysts of *T. gondii*; previously, cysts were detected in chicken and horse from elsewhere in México (Dubey et al., 2004; Alvarado-Esquivel et al., 2012, 2015). Therefore, feeding wild animals in captivity with raw meat may represent a high risk factor of infection by *T. gondii* (Alvarado-Esquivel et al., 2013). Freezing meat at −12 °C for at least 7 days before feeding it to animals is considered to be the most practical strategy to reduce *T. gondii* infection in carnivores (Dubey, 2010).

Almost all felines were positive, while most mouflons were negative;

these results seem to be interesting. It is important to note that all wild felines are old adults (some of them older than 20 years) and throughout their lives they have been constantly exposed to tissue cysts or oocysts of the parasite, unlike mouflons, which are mainly young adults (under 5 years) and are only exposed to the risk of consuming *T. gondii* oocysts. Alternatively, seronegative mouflons could be infected with *T. gondii*, but their antibody titers could be below the ELISA detection limit; this may be due to fluctuations in immunoglobulin levels and they have not been re-infected with the parasite or the parasites cyst in the tissues of the mouflons have not been reactivated (Rougier et al., 2017). This is supported by the fact that some mouflons when retested a year later became seronegative while others remained seropositive (green and blue dots in Fig. 1C, respectively).

By conventional anatomopathological techniques, no lesions associated to acute or chronic infection of *T. gondii* were identified in sampled animals, which was congruent with lack of clinical manifestations related to *T. gondii* infection in any of the positive animals. Some immuno-positive small structures were found in the spleen and the muscle of one mouflon and in the brain of one lion by IHC (L2). In clinically healthy animals, it is difficult to find lesions associated with *T. gondii* infection, thus more sensitive tests, such as IHC and PCR, are required to demonstrate the presence of this infectious agent (Silva et al., 2013).

Surface antigens *SAG1, Alt. SAG2* and *SAG3* were the genes with the highest amount of positive and genotyped results. This correlates with results previously obtained by our group using clinical samples of humans (mother/newborn pairs), feral cats and stray dogs (Rico-Torres et al., 2018; Valenzuela-Moreno et al., 2019, 2020). Our interest was to genotype *T. gondii* from clinical samples; therefore, the success in the genetic characterization of this parasite was limited but with interesting results, since we were able to identify a triple infection in the brain of mouflon 1: I + II + III at *SAG3* gene. The detection of mixed infections is of particular interest in epidemiological studies, since they demonstrate the circulation of several strains in a specific region that can result in the recombination and generation of new variants when they are simultaneously ingested by felines. In México, the presence of more than one strain in a given host has become rather common, because it has been described in animals from neo-tropical regions of México, i.e. in feral cats from Quintana Roo, stray dogs from Chiapas and pigs from Yucatán (Cubas-Atienzar et al., 2018; Valenzuela-Moreno et al., 2019, 2020). However, this phenomenon is not exclusive to those regions, since in human congenital cases from the Valley of Mexico, and now in mouflons from Puebla (both neo-artic regions) we have found mixed infections as well (Rico-Torres et al., 2018).

In conclusion, we found a high infective pressure of *T. gondii* in the collection, supported by changes in its prevalence in European

Table 2
Serology and Multiplex multilocus genotyping of *Toxoplasma gondii* from mouflons (*Ovis musimon*) and lions (*Panthera leo*) from Puebla, México.

ID	Tissue	IgG Abs[a]	B1 gene hnPCR[b]	529 bp repeat PCR	Tachyzoites/mg tissue	Genetic markers									
						SAG1	altSAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	Apico
Mouflon 1	Brain	+	+	+	<50	II or III	I	I + II + III							I
Mouflon 2	Muscle	+	+	+	<50	(+)	III				(+)			(+)	(+)
Lion 1	Liver	+	+	+	nd	II or III		III	(+)						(+)
Lion 1	Spleen	+	+	+	–										
Lion 2	Liver	+	+	+	nd	(+)	I	III	(+)						

[a] Anti-*T. gondii* antibodies by ELISA.
[b] Heminested PCR. (+): positive sample to *T. gondii* but insufficient DNA amount to determine RFLP pattern. nd: not determined due to insufficient DNA. Empty cells of genetic markers imply no amplicons were obtained by nested PCR.

mouflons; also, and as expected, the prevalence of infection by *T. gondii* in felines was high. It was also supported by the finding of at least four parasites variants among herbivores and carnivores, as well as a triple infection in one mouflon.

Declaration of competing interest

None.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.07.005.

References

Alvarado-Esquivel, C., Alvarado-Esquivel, D., Dubey, J.P., 2015. Prevalence of *Toxoplasma gondii* antibodies in domestic donkeys (*Equus asinus*) in Durango, Mexico slaughtered for human consumption. BMC Vet. Res. 11, 6.

Alvarado-Esquivel, C., Gayosso-Domínguez, E., Villena, I., Dubey, J.P., 2013. Seroprevalence of *Toxoplasma gondii* infection in captive mammals in three zoos in Mexico City, Mexico. J. Zoo Wildl. Med. 44, 803–806.

Alvarado-Esquivel, C., Rodríguez-Peña, S., Villena, I., Dubey, J.P., 2012. Seroprevalence of *Toxoplasma gondii* infection in domestic horses in Durango State, Mexico. J. Parasitol. 98, 944–945.

Aubert, D., Ajzenberg, D., Richomme, C., Gilot-Fromont, E., Terrier, M.E., de Gevigney, C., Game, Y., Maillard, D., Gibert, P., Dardé, M.L., Villena, I., 2010. Molecular and biological characteristics of *Toxoplasma gondii* isolates from wildlife in France. Vet. Parasitol. 171, 346–349.

AZA Nutrition Advisory Group, 2016. Body condition scoring resource center. https://nagonline.net/wp-content/uploads/2016/08/Lions-Dikeman-Felid-TAG.pdf. Accessed on June 1, 2020.

AZA Nutrition Advisory Group, 2017. Body condition scoring resource center. https://nagonline.net/wp-content/uploads/2017/03/bcs-sheep.pdf. Accessed on June 1, 2020.

Caballero-Ortega, H., Quiroz-Romero, H., Olazarán-Jenkins, S., Correa, D., 2008. Frequency of *Toxoplasma gondii* infection in sheep from a tropical zone of Mexico and temporal analysis of the humoral response changes. Parasitology 135, 897–902.

Caballero-Ortega, H., Castillo-Cruz, R., Murieta, S., Ortíz-Alegría, L.B., Calderón-Segura, E., Conde-Glez, C.J., Cañedo-Solares, I., Correa, D., 2014. Diagnostic-test evaluation of immunoassays for anti-*Toxoplasma gondii* IgG antibodies in a random sample of Mexican population. J. Infect. Dev. Ctries. 8, 642–647.

Cedillo-Peláez, C., 2015. Diversidad genética de *Toxoplasma gondii* en animales domésticos y fauna silvestre de México (Tesis de Doctorado en Ciencias). Universidad Nacional Autónoma de México., Ciudad de México.

Cedillo-Peláez, C., Rico-Torres, C.P., Salas-Garrido, C.G., Correa, D., 2011. Acute toxoplasmosis in squirrel monkeys (*Saimiri sciureus*) in Mexico. Vet. Parasitol. 180, 368–371.

Cubas-Atienzar, A.I., Hide, G., Jiménez-Coello, M., Ortega-Pacheco, A., Smith, J.E., 2018. Genotyping of *Toxoplasma gondii* from pigs in yucatan, Mexico. Vet. Parasitol. Reg. Stud. Reports 14, 191–199.

de Camps, S., Dubey, J.P., Saville, W.J.A., 2008. Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoos in the midwestern United States. J. Parasitol. 94, 648–653.

Dubey, J.P., 2010. Toxoplasmosis of Animals and Humans, second ed. CRC Press, Boca Raton, Florida.

Dubey, J.P., Morales, E.S., Lehmann, T., 2004. Isolation and genotyping of *Toxoplasma gondii* from free-ranging chickens from Mexico. J. Parasitol. 90, 411–413.

Espinosa-Avilés, D., Martínez-Morales, M.S., 2007. An outbreak of toxoplasmosis in a colony of captive squirrel monkey (*Saimiri sciureus*). Vet. Mex. 38, 365–369.

Ferreira, S.C.M., Torelli, F., Klein, S., Fyumagwa, R., Karesh, W.B., Hofer, H., Seeber, F., East, M.L., 2019. Evidence of high exposure to *Toxoplasma gondii* in free-ranging and captive African carnivores. Int. J. Parasitol. Parasites Wildl. 8, 111–117.

Grigg, M.E., Sundar, N., 2009. Sexual recombination punctuated by outbreaks and clonal expansions predicts *Toxoplasma gondii* population genetics. Int. J. Parasitol. 39, 925–933.

Hejlicek, K., Literak, I., Nezval, J., 1997. Toxoplasmosis in wild mammals from the Czech Republic. J. Wildl. Dis. 33, 480–485.

Homan, W., Vercammen, M., 2000. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. Int. J. Parasitol. 30, 69–75.

INEGI, 2020. https://www.inegi.org.mx/app/areasgeograficas/?ag=21114.

Olamendi-Portugal, M., Caballero-Ortega, H., Correa, D., Sánchez-Alemán, M.A., Cruz-Vázquez, C., Medina-Esparza, L., Ortega-S, J.A., Cantu, A., García-Vázquez, Z., 2012. Serosurvey of antibodies against *Toxoplasma gondii* and *Neospora caninum* in white-tailed deer from Northern Mexico. Vet. Parasitol. 189, 369–373.

Philippa, J.D.W., Martina, B.E.E., Kuiken, T., Van de Bildt, M.W.G., Osterhaus, A.D.M.E., Leighton, F.A., Daoust, P.Y., Nielsen, O., Pagliarulo, M., Schwantje, H., Shury, T., van Herwijnen, R., 2004. Antibodies to selected pathogens in free-ranging terrestrial carnivores and marine mammals in Canada. Vet. Rec. 155, 135–140.

Pujol-Riqué, M., 1999. Design of a one-tube hemi-nested PCR for detection of *Toxoplasma gondii* and comparison of three DNA purification methods. J. Med. Microbiol. 48, 857–862.

Rico-Torres, C.P., Valenzuela-Moreno, L.F., Luna-Pastén, H., Figueroa-Damián, R., Gómez-Toscano, V., Hernández-Delgado, L., Escobedo-Torres, M.P., Correa, D., 2018. High heterogeneity, mixed infections and new genotypes in human congenital toxoplasmosis cases in the mega-metropolis of Central Mexico. Acta Trop. 178, 124–129.

Robert-Gangneux, F., Dupretz, P., Yvenou, C., Quinio, D., Poulain, P., Guiguen, C., Gangneux, J.P., 2010. Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. Pediatr. Infect. Dis. J. 29, 33–38.

Rougier, S., Montoya, J.G., Peyron, F., 2017. Lifelong persistence of *Toxoplasma* cysts: a questionable dogma? Trends Parasitol. 33, 93–101.

Schunemann, M.A., Constantino, C.F., 2002. Técnicas de necropsia en animales domésticos - 2: edicion (Spanish Edition). Manual Moderno 110.

Sedlák, K., Bártová, E., 2006. Seroprevalences of antibodies to *Neospora caninum* and *Toxoplasma gondii* in zoo animals. Vet. Parasitol. 136, 223–231.

Silva, A.F., Oliveira, F.C.R., Leite, J.S., Mello, M.F.V., Brandão, F.Z., Leite, R.I.J.C.K., Frazão-Teixeira, E., Lilenbaum, W., Fonseca, A.B.M., Ferreira, A.M.R., 2013. Immunohistochemical identification of *Toxoplasma gondii* in tissues from modified agglutination test positive sheep. Vet. Parasitol. 191, 347–352.

Su, C., Shwab, E.K., Zhou, P., Zhu, X.Q., Dubey, J.P., 2010. Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitology 137, 1–11.

Valenzuela-Moreno, L.F., 2014. Frecuencia y genotipificación de *Toxoplasma gondii* en carnívoros y herbívoros silvestres de una colección privada. (Tesis de Maestría). México, D.F. México. Universidad Nacional Autónoma de México.

Valenzuela-Moreno, L.F., Rico-Torres, C.P., Cedillo-Peláez, C., Luna-Pastén, H., Méndez-Cruz, S.T., Lara-Martínez, G., Correa, D., Caballero-Ortega, H., 2019. Mixed *Toxoplasma gondii* infection and new genotypes in feral cats of Quintana Roo, México. Acta Trop. 193, 199–205.

Valenzuela-Moreno, L.F., Rico-Torres, C.P., Cedillo-Peláez, C., Luna-Pastén, H., Méndez-Cruz, S.T., Reyes-García, M.E., Correa, D., Alves, B.F., Pena, H.F.J., Caballero-Ortega, H., 2020. Stray dogs in the tropical state of Chiapas, Mexico, harbour atypical and novel genotypes of *Toxoplasma gondii*. Int. J. Parasitol. 50, 85–90.

Yang, Y.R., Feng, Y.J., Lu, Y.Y., Dong, H., Li, T.Y., Jiang, Y.B., Zhu, X.Q., Zhang, L.X., 2017. Antibody detection, isolation, genotyping, and virulence of *Toxoplasma gondii* in captive felids from China. Front. Microbiol. 8, 1414.