**Toxoplasma gondii** in feral American minks at the Maullín river, Chile

Maximiliano A. Sepúlveda\(^a,b,\)\(^*\), Claudia Muñoz-Zanzi\(^a,c\), Carla Rosenfeld\(^a\), Rocio Jara\(^a\), Katharine M. Pelican\(^b\), Dolores Hill\(^d\)

---

* Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja s/n, Casilla 567, Valdivia, Chile
* Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN 55108, USA
* Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, 1300 S Second St. Suite 300, Minneapolis, MN 55454, USA
* United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Beltsville, MD 20705, USA

**A R T I C L E  I N F O**

Article history:
Received 30 October 2008
Received in revised form 4 August 2010
Accepted 20 September 2010

**Keywords:**
American mink
Chile
Neovison vison
PCR
Toxoplasma gondii
Seroprevalence

**A B S T R A C T**

American mink (*Neovison vison*) is a widely distributed invasive species in southern Chile. Thirty four feral minks were trapped at two distinct sites (rural and peri-urban), diet analyzed and *Toxoplasma gondii* exposure compared using PCR and specific antibodies. Serum samples were evaluated using a commercial latex agglutination test where a titer \(\geq 1:32\) was considered positive. Of 30 mink analyzed, 21 (70%) were positive to *T. gondii* antibodies, with titers ranging from 1:32 to 1:2048. As expected, adult mink showed higher seroprevalence of exposure to *T. gondii* (18/21) than young mink (3/9) \((P = 0.008)\). There was not statistically significant difference between sex groups \((P = 0.687)\). Differences in seroprevalence were observed between the two sample sites with a higher proportion of positive individuals in the peri-urban area, and therefore, closer to human settlements (35.7% vs. 100%, \(P = 0.0001\)). Individuals positive to *T. gondii* using PCR and/or serology showed similar differences by site with higher infected individuals in peri-urban areas (58.8% vs. 100%, \(P = 0.007\)). Diet of American mink based in fecal composition analyses was mainly based on crustaceans (frequency of occurrence: crustaceans = 100%, birds and rodents < 7%), suggesting that the high observed prevalence of *T. gondii* infection might be more associated with its aquatic behavior (e.g. ingestion of oocysts in contaminated fresh water) than with their trophic behavior (e.g. preying over species that can have *T. gondii* cysts in their tissues). As an invasive species potentially subject to routine culling to maintain population sizes under control, minks could be used as a sentinel species to monitor pathogens of public and wildlife health importance, such as *T. gondii*, in aquatic environments.

© 2010 Elsevier B.V. All rights reserved.

---

1. **Introduction**

The American mink (*Neovison vison*), a mustelid carnivore species, was first brought to Chile in the 1930s for the pelt industry. In the 1970s feral populations of mink farm escapees were first reported (*Servicio Agrícola y Ganadero, 1988*). At present, the species is considered an invasive species and is widely distributed throughout southern Chile including Tierra del Fuego Island (38°–35°S to 55°S) and adjacent archipelagos (*Rozzi and Sherriffs, 2003*). Research on the American mink in this neotropical area has focused mainly on diet, distribution, and aspects of interspecific competition and predation (*Medina, 1997; Jakse et al., 2002; Rozzi and Sherriffs, 2003; Schuttler et al., 2008; Fasola et al., 2009; Ibarra et al., 2009*). Monitoring of specific pathogens in free-ranging carnivores can provide estimates of environmental contamination because of the high risk of...
exposure associated with their trophic position (Smith and Frenkel, 1995). However, there are no published studies on the infections present in feral mink populations in South America.

The protozoan parasite Toxoplasma gondii is a widespread pathogen of warm-blooded animals, including humans. This parasite has a two-host life cycle. Felids are the definitive hosts and the only animals known to shed oocysts in their feces. They can be infected through oocyst exposure or consumption of infected intermediate hosts. Many animals, including mice, birds, domestic livestock and humans can serve as intermediate hosts, where invasive stages of T. gondii may spread throughout the muscles, nervous system and other tissues, forming long-lived tissue cysts (Frenkel and Dubey, 1972). Intermediate hosts can be infected via ingestion of feline fecal matter contaminated with sporulated oocysts, ingestion of tissue cysts or tachyzoites in meat from infected animals, or congenitally due to maternal infection during gestation (Dubey and Beattie, 1988).

In immune competent humans, acute infection with T. gondii is usually asymptomatic or causes mild disease; however, it can cause devastating disease in congenitally infected children and in people with depressed immunity. Similarly, clinical and subclinical toxoplasmosis has been reported in many host wild species and serologic surveys indicate that T. gondii infections are common in free-ranging carnivores (Hill et al., 2005). The critical role of T. gondii in mortality of southern sea otters (Enhydra lutris nereis) (Kreuder et al., 2003), where it is considered a significant cause of encephalitis (Cole et al., 2000; Lindsay et al., 2001; Miller et al., 2004), is of particular relevance for this study. Miller et al. (2002) has also documented a high prevalence of infection in sea otters and concurrent population declines along the Pacific coast of the U.S., probably the result of surface water run-off contaminated with T. gondii oocysts.

The objective of this study was to describe T. gondii infection in American mink in Chile and to identify factors associated with infection, most importantly, proximity to human populations. Secondary objectives included description of dietary aspects and abundances.

2. Material and methods

2.1. Study area

All mink captures were located within the Maullín river basin of the Llanquihue Province, Lake District in Chile (41°26′S; 73°07′W). The basin is characterized by mesotemplate hyper-humid climate, with yearly average precipitation of 2021 mm and average temperature of 10.7 °C (Luebert and Pliscoff, 2006). The vegetation associated with the river is primarily swamp forest which is characterized by native species such as Myrceugenia exsucca, Blepharocalyx cruckshanksii, Luma gayana, Drimys winteri, Tepualia stipularis, Blechnum chilense, Chusquea quila, Boguila trifoliata and Cissus striata (Hauenstein et al., 2005). The river adjacent area is mostly used for agricultural purposes such as livestock and dairy farming. The total surface of the Maullín basin covers 397,200 ha with a total human population of 273,256 inhabitants (INE, 2002). Within this basin, one peri-urban site and one rural site were selected for mink captures. The peri-urban site was located 3.2 km from Puerto Varas (22,309 habitants), 7.6 km from Llanquihue (12,728 habitants) and 12 km from Puerto Montt (155,895 habitants). The rural site was located 16.2 km, 19.3 km and 17.3 km, respectively, from the previously mentioned urban areas, as well as 23.1 km from Los Muermos (5,707 habitants) (INE, 2002) (Fig. 1).

2.2. American minks

American minks were trapped between March and October of 2007 using Tomahawk traps. Eighteen traps per site were distributed homogeneously over a 3.8 km area in the peri-urban site and a 2 km area in the rural site. Captured minks were anaesthetized with a combination of Ketamine (Imalgene®, 10 mg/kg; Merial) and Xylazine (Rompun®, 0.5 mg/kg; Bayer). Blood samples for serum were collected by cardiac puncture and stored at −20 °C until testing. Data collected included sampling site, age, sex, weight, and length. Age category was estimated by teeth condition as follows: juvenile (adult teeth without abrasion and tartar) or adult (teeth abraded and with tartar) (modified from Fournier-Chambrión et al., 2004).

Body condition or relative weight (K) was estimated for adult individuals only as $K = W/(aL^n)$, where $W$ = weight (g), $L$ = body length (cm), and $a$ and $n$ are constants (Kruuk and Conroy, 1991). Values for males were $a = 0.0252$ and $n = 2.8833$ and values for females were $a = 0.0524$ and $n = 2.6594$ (Sidorovich et al., 1999). K values close to 1 indicate adequate body condition, less than 1 indicate poor body conditions, and values higher than 1 indicate overweight (Kruuk, 1995). After data collection and under sedation, all individuals were sacrificed by cardiac puncture with a commercial euthanasia solution (T61®; Intervet, Canada). Brain tissue was harvested and stored at −20 °C for PCR testing. The National Agricultural and Livestock Service (Servicio Agrícola y Ganadero) from Chile provided the required capture permit for this research (legal permit no. 3206 from July 14, 2006 and its modification no. 904 from February 23, 2007).

2.3. T. gondii antibody detection

Samples were evaluated for IgG antibodies against T. gondii using a commercial latex agglutination test (Toxotest-MT®, Eiken Chemical Company, Ltd, Tokyo, Japan) performed at the Preventive Medicine Institute laboratory, Universidad Austral de Chile following the manufacturer’s protocol. Briefly, this test uses inactivated T. gondii antigen coated latex particles that agglutinate when exposed to T. gondii specific antibodies. Sera were diluted in duplicate wells from 1:16 to 1:2408 in buffer solution to determine antibody titers. Based on the manufacturer’s recommendation, a titer ≥1:32 was considered positive. A positive result is indicative of past exposure to T. gondii. In order to increase reliability of results, all samples were re-tested at the Minnesota Veterinary Diagnostic Laboratory using a similar latex agglutination test and interpretation.
2.4. *T. gondii* DNA detection

DNA was extracted from all available brain tissues using a commercial kit (DNeasy® Blood and Tissue Blood and Tissue, Qiagen Inc., Valencia, CA) and following the manufacturer’s protocol. Extracted DNA was shipped to the Animal Parasitic Diseases Laboratory of USDA-ARS on FTA® cards (Whatman Inc., Kent, UK) for PCR testing using a published protocol (Hill et al., 2006). PCR was performed using direct and semi-nested methods to amplify segments of the 35-fold repetitive DNA region B1 of *T. gondii*. Direct PCR was performed on individual samples using the primer pair forward: 5′-GGAACTGCATCCGTTCATGAG-3′ and reverse: 5′-TCTTTAAAGCGTTCGTGGTC-3′. Semi-nested PCR was performed using 1 ml of the amplicons resulting from the first round direct PCR as the target DNA, the forward primer 5′-TGCACTAGTTGCAGTCACTG-3′, and the reverse primer from the first round PCR. Cycling conditions for both the direct and semi-nested PCR were denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min. Products from the direct and semi-nested PCR were analyzed on pre-poured 2.0% agarose E-gels along with Hae III DNA markers (Invitrogen, Carlsbad, CA).

2.5. Mink diet and abundances

Fecal samples were collected from the same transects used for mink trapping. Mink feces were recognized based on a combination of size, appearance and the presence of stray hairs ingested while grooming (Chehébar and Benoit, 1988; Bonesi et al., 2004). After collection, feces were washed, passed through a sieve, dried and analyzed under the microscope. Undigested prey remains were classified using reference material obtained at the Zoology Institute of the Universidad Austral de Chile, expert consults and keys from Retamal (1981) for crustaceans and Chehébar and Martin (1989) for mammals. The diet was described as: (1) the prey occurrence (the number of feces in which the prey class was identified) and (2) frequency of occurrence (number of occurrences of a prey class in relation to the sum of occurrences of all prey classes).

Relative abundances of mink in the study area were inferred by trapping (Bonesi et al., 2004) while calibrating the difference in sampling effort between the two sites (rural and urban). Sampling effort was calculated as number of traps night/number of minks trapped.

2.6. Statistical analysis

Seroprevalence of *T. gondii* infection was defined as the proportion of individuals with a positive ELISA result. Because of the known likelihood of false negative results with PCR (Hill et al., 2006) an overall estimation of prevalence of infection was also calculated defining infected individuals as those who were positive to either *T. gondii*-specific antibodies or PCR. Confidence intervals (CI) for the estimates were calculated using exact methods. Prevalences were compared by location (rural, peri-urban), age class (adult, juvenile), and sex using Fisher’s exact test.
Table 1
Results of risk factors associated with T. gondii antibodies in serum samples from N. vison using agglutination in latex test in Maullín river, Chile.

<table>
<thead>
<tr>
<th>Location</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Total (%)</th>
<th>OR (95% IC)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peri-urban</td>
<td>16 (100%)</td>
<td>0 (0%)</td>
<td>16 (53.3%)</td>
<td>NC</td>
<td>P = 0.0001*</td>
</tr>
<tr>
<td>Rural</td>
<td>5 (35.7%)</td>
<td>9 (64.3%)</td>
<td>14 (46.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (73.67%)</td>
<td>5 (26.3%)</td>
<td>19 (63.3%)</td>
<td>1.6 (0.32–7.9)</td>
<td>P = 0.687*</td>
</tr>
<tr>
<td>Female</td>
<td>7 (63.6%)</td>
<td>4 (36.4%)</td>
<td>11 (36.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>17 (81%)</td>
<td>4 (19%)</td>
<td>21 (70%)</td>
<td>4.9 (0.9–30)</td>
<td>P = 0.081*</td>
</tr>
<tr>
<td>Juvenile</td>
<td>4 (44.4%)</td>
<td>5 (55.6%)</td>
<td>9 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21 (70%)</td>
<td>9 (30%)</td>
<td>30 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NC: not calculated.
* Fisher exact test.

Table 2
Distribution of T. gondii antibody titers (<16, 32, 64, 128, 256, 512, 1024, 2048) by age group and location among American minks from Maullín river, Chile.

<table>
<thead>
<tr>
<th>Titer dilution</th>
<th>Total</th>
<th>Rural</th>
<th>Peri-urban</th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1:16</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>1:16</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1:32</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1:64</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1:128</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1:256</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>1:512</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1:1024</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1:2048</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Stratified analysis was used to adjust for age and for detecting effect modification (Agresti, 2002). In addition, t-test was used to compare body condition score (K) between positive and negative animals and Pearson correlation coefficient was used to examine a relation between ln(antibody titer) against T. gondii in positive individuals and body condition score. Due to the small sample size, for all analysis, a P < 0.1 was considered statistically significant. All analyses were performed using R 2.4.1 (R Development Core Team, 2005).

3. Results

A total of 34 American minks (17 individuals at each site) were captured, using 270 and 188-traps night at the rural and peri-urban sites, respectively. Relative abundance calculated by capture and removal was 8.5 minks/km in the rural site and 4.5 minks/km at the peri-urban site. All animals appeared to be healthy on physical examination. Serum samples were obtained from 30 mink, 16 from the peri-urban site and 14 from the rural site, and 21 were classified as seropositive for T. gondii (Table 1) by both laboratories. Serum titers for seropositive animals ranged from 1:32 to ≥1:2048 (Table 2). Overall seroprevalence was 70% (90% CI: 53.5–83.4%). Serostatus differed (P = 0.08) with age where 81.0% (17/21) of adults were seropositive compared to 44.4% (4/9) of juveniles. The age distribution of captured mink differed by site where 36% (5/14) of minks in the rural site were adults and 100% (16/16) of minks in the peri-urban site were adults. Seroprevalence in minks from the rural site was 35.7% (5/14). In contrast, all 16 individuals (100%) from the peri-urban site were classified as seropositive (P = 0.001). Because of this markedly different age distribution, the true association between site and serostatus was confounded by age. In order to overcome this limitation, the association between site and serostatus was examined among adults only which resulted in the same type of positive association between peri-urban site and seroprevalence. Considering adults only, seroprevalence was 100% (16/16, 90% CI: 82.9–100%) for the peri-urban site and 20% (1/5, 90% CI: 1–65.7%) for the rural site (P = 0.0008). Considering either seropositive or PCR positive as an indication of T. gondii infection, adults were more likely to be infected (19/22 = 86.3%) compared with juveniles (7/11 = 63.6%), though not statistically significant (P = 0.18). Similarly to serology only results, animals from the peri-urban site (16/16 = 100%) were more likely to be infected compared with animals from the rural site (10/17 = 82%, P = 0.007), even controlling by age (P = 0.012).

Among adults, body condition score ranged from 0.33 to 1.10 with a mean of 0.82. Seropositive animals (mean K = 0.89) had a statistically significant higher score than seronegative animals (mean K = 0.54) (P = 0.0099). However, there was no correlation between antibody titers and body condition score (r = 0.25, P = 0.33). There was no statistically significant association between serostatus and sex (P = 0.687) (Table 1).

In relation to mink’s diet, sixty fecal samples were collected and the main diet item present in all feces was crustaceans, other items represented in less than 7% frequency occurrence were mammals, birds and fish (Table 3).

4. Discussion

To our knowledge this is the first study of T. gondii prevalence in feral minks over a small spatial and temporal scale. Previous reports were based on samples collected from larger regions and over long time intervals (Tizard et al., 1976; Smith and Frenkel, 1995). Even though the number of tested individuals included in our study was limited, we
observed a significant difference in exposure to *T. gondii* between the peri-urban and rural sites. This difference was striking considering the relatively short distances between the two sites (15 km). As expected with serologic results and chronic infections, we observed that increasing mink age increased the odds of positivity to *T. gondii* and chronic infections, we observed that increasing mink age increased the odds of positivity to *T. gondii* probably as a result of higher probability of life time exposure in older animals. In the case of sex, we did not find a difference in the seroprevalence of *T. gondii* antibodies in mink. If that is the case, it could indicate that both male and female have similar probabilities of acquiring infection with *T. gondii*, despite the known differences in spatial ecology and movements between sexes in these mustelids (Dunstone, 1993). Diet plays a key role in *T. gondii* transmission. Previous studies of *T. gondii* in mammals have reported higher prevalences in carnivore species than omnivores and herbivores (Smith and Frenkel, 1995; Hejílcek et al., 1997; Sobrino et al., 2007). The diet of the American mink is composed of a wide range of prey including small mammals and birds (Dunstone, 1993). In this particular region, the American mink’s diet is composed mainly of crustaceans and secondarily by rodents and fish (Ruiz et al., 1996; Medina, 1997). This is consistent with what was observed in the feces of minks trapped in this study. The role of crustaceans in the transmission of *T. gondii* oocysts is unknown, however, oocysts have been observed in some species such as bivalves (Miller et al., 2008). Considering the importance of crustaceans in the diet of American mink in this region, future research should assess this potential route of transmission.

Another major route of exposure for *T. gondii* is ingestion of oocysts in the environment. The habitat of American mink is highly associated with water courses (Dunstone, 1993); therefore, ingestion of oocysts directly from contaminated water (Dubey, 2004) might be explaining the high prevalence of *T. gondii* observed in mink. This is consistent with findings in other semi-aquatic and aquatic species like the California sea otter. Given the apparent minimal exposure of these mink to known intermediate hosts, environmental exposure seems likely. This is an important aspect to consider in future research.

Toxoplasmosis studies in humans and domestic animals show that infection in Chile is highly prevalent and endemic. Seroepidemiology of *T. gondii* in the human population of Chile showed significant higher levels of seropositivity in urban [38.0%] vs. rural areas [33.5%] and southern areas vs. northern areas (Contreras et al., 1996). Furthermore, a recent study estimated that about half of recent human infections were associated with oocyst ingestion (Muñoz-Zanzi et al., 2010). These results are consistent with our observations of high prevalence of infection in minks and its association with populated areas which could be explained by higher abundances of cats in urban or peri-urban areas than in rural areas. The humid environmental conditions in this southern part of Chile are also favorable for the survival of the oocysts in the environment. At present, there have been no studies on cat ecology and management in the south of Chile. Such studies could help understand the epidemiology of *T. gondii* in this ecosystem, including the inter-relationships between the urban and wildlife cycles.

### Acknowledgements

The authors wish to thank Ignacio Rodriguez, Jorge Ruiz, and Tatiana Probsto for logistic support and the landowners of the trapping sites. This project was financed by personal funds (M.A.S.), Universidad Austral de Chile Graduate School, the Southern River Otter Project CODEFF-FZS and a Fulbright-CONICYT scholarship (M.A.S.).

### References


### Table 3

<table>
<thead>
<tr>
<th>Prey class</th>
<th>O</th>
<th>FO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Samasthacus spinifrons</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Aegla spp.</td>
<td>13</td>
<td>21.7</td>
</tr>
<tr>
<td>Mammals</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Abrothrix longipilis</em></td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>Fish</td>
<td>2</td>
<td>3.3</td>
</tr>
</tbody>
</table>