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[***TOXOPLASMA GONDII* PREVALENCE, PARTIAL GENOTYPES, AND SPATIAL VARIATION IN NORTH AMERICAN RIVER OTTERS (*LONTRA CANADENSIS*) IN THE UPPER PENINSULA OF MICHIGAN, USA**](https://doi.org/10.7589/jwd-d-22-00021)

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**ABSTRACT:** *Toxoplasma gondii* is a ubiquitous parasitic protozoan that poses a health threat to wildlife and human health worldwide. Oocysts shed into the environment in felid host feces may persist for several years. Runoff from rainfall and snowmelt may carry the oocysts into waterways. Semiaquatic mammals such as the Northern American river otter (*Lontra canadensis*) are particularly at risk of exposure, as they may encounter infective stages in both terrestrial and aquatic environments. Despite this risk, only a small number of studies have examined the prevalence of *T. gondii* in US river otter populations. Tongue tissue was sampled from 124 otters from the Upper Peninsula of Michigan submitted by trappers to the Michigan Department of Natural Resources in the 2018–19 harvest season. Following DNA extraction, a portion of the B1 *T. gondii* gene was amplified with PCR. A subset of positive samples was genotyped for comparison with known *T. gondii* sequences. Of the 124 tongue samples, 35 (28%) were positive for *T. gondii*. Prevalence did not differ significantly between sexes or age classes across the entire study area. Most (53.8%) of the genotyped samples were type 4 (type 12), a genotype commonly found in North American wildlife. Genotypes 127 and 197 were also found. Three clusters of *T. gondii* prevalence were identified through SaTScan analysis, although they were not significant. When modeling prevalence of *T. gondii* with covariates at individual otter locations, the top three models included the presence of *Sarcocystis*, area of exotic plants, area of agriculture, and sex of the otter. Our results suggest that *T. gondii* is widespread in otter populations in the Upper Peninsula of Michigan.

**Key Points:**

* *Toxoplasma gondii* - zoonotic protozoan parasite
	+ May cause encephalitis, abortions, stillbirths, and death in humans
	+ Shed oocytes become infective after sporulation in 1-5 days, viable for several years
	+ Hydrophilic covering and buoyancy may yield extended life even in aquatic environments
	+ Several studies show a higher seroprevalence in aquatic and semiaquatic species due to increased exposure risk
* NARO prevalence in Northern Michigan was significantly lower than terrestrial mammals assessed in that area, but similar to other semiaquatic animals assessed
	+ Similar to the prevalence of *Toxoplasma gondii* in NAROs in NC
* Results may be biased as the samples were only from trapped individuals
* No sex or age predilection
	+ Previous studies have had variable sex predilection, and almost always higher prevalence in older otters, suspect this is a skew due to trapping
* Most often Genotype 4 which is the genotype found most often in NA wildlife
* Significantly higher incidence in watersheds draining to Lake Superior compared to Lake Michigan/Huron, possibly due to increased snow loads
* Increased prevalence of *Toxoplasma gondii* with presence of *Sarcocystis* spp.
* Increased prevalence of *Toxoplasma gondii* in areas with exotic plants or agriculture
	+ May indicate human disturbances

**Related Articles:**

Sanders CW, Olfenbuttel C, Pacifici K, Hess GR, Livingston RS, DePerno CS. LEPTOSPIRA, PARVOVIRUS, AND TOXOPLASMA IN THE NORTH AMERICAN RIVER OTTER (LONTRA CANADENSIS) IN NORTH CAROLINA, USA. *J Wildl Dis*. 2020;56(4):791-802.

**EVALUATING BAITS WITH LUFENURON AND NITENPYRAM FOR FLEA CONTROL ON PRAIRIE DOGS (CYNOMYS SPP.) TO MITIGATE PLAGUE.** JWD 2023. Eads DA, Castle KT, Wild MA, Borchert JN, Livieri TM, Matchett MR, Dobesh P, Hughes JP, Childers E.

Abstract: Plague, caused by Yersinia pestis, is a widespread threat to endangered black-footed ferrets (*Mustela nigripes*) and their primary prey, prairie dogs (*Cynomys spp.*). Wildlife biologists most commonly manage plague using insecticides to control fleas, the primary vectors of Y. pestis. We tested edible baits containing the insecticides lufenuron and/or nitenpyram in prairie dogs. During a laboratory study, we treated 26 white-tailed prairie dogs (*Cynomys leucurus*) with lufenuron at 300 mg/kg body mass. All animals remained clinically healthy over the 9 wk monitoring period. Although serum lufenuron concentrations were >130 ppb in two treatment groups at week 1, concentrations declined to ≤60 ppb after 3 wk in non-torpid prairie dogs and after 7 wk in torpid prairie dogs. In a field experiment, we tested baits containing a combination of 75 mg lufenuron and 6 mg nitenpyram, respectively, in black-tailed prairie dogs (*Cynomys ludovicianus*). We uniformly distributed baits at 125 baits/ha on two plots (treated once) and 250 baits/ha on two plots (each treated twice 4.4 wk apart). Following treatments, flea abundance increased on prairie dogs and remained stable in burrows. Our findings indicate that **baits containing lufenuron and nitenpyram, at the reported treatment rates, are ineffective tools for flea control on prairie dogs.** Future experiments might evaluate efficacy of higher doses of lufenuron and nitenpyram, and repetitive treatments at differing intervals over time to evaluate potentially therapeutic treatments.

Background:

* Plague = fatal zoonosis caused by flea-borne bacterium Yersinia pestis
* Threat to endangered BFF and their primary prey, prairie dogs (BTPD)
* Fipronil (GABA receptor antagonist) baits (FipBait formulation) incorporated into several species conservation plans for control of fleas
	+ Field studies in P. dogs proved effective control for 12-24 months in most cases
* Lufenuron and nitenpyram have long history use in domestic mammals and high safety margin
	+ Cat lufenuron: 30 mg/kg q30 days maintains 50-100 ppb serum conc effective for fleas
* Goal of study to find fipronil alternative to fipronil to flea control in PD in case of resistance

Methods/Results: (I) Tested PO lufenuron in torpid and non-torpid PDs, (II) conducted field experiment with lufenuron-nitenpyram bait (LNBits) in PDs, and (III) evaluated lab data from PDs treated with PO nitenpyram/exposed to fleas

* Part (I):  n=30 PD, 300 mg/kg(!) lufenuron PO, some in torpor (induced) and some non-torpid
	+ Torpor = lack of reaction to stimulus (tested by sprinkling sawdust on them)
	+ Serum levels q2 weeks (under GA) x4 via high pressure liquid chromatography
	+ **Results:** serum lufenuron concentrations similar for non-torpid and torpid groups 1 week post-treatment, but thereafter concentrations were significantly higher in the torpid group
* Part (II): placed hundreds of baits (every 9 m) on multiple plots ofTPD territory in S. Dakota
	+ Sampled fleas on PD before and after baiting: trapped, GA, flea comb, release
	+ Sampled n=30 burrows: swabbed with plumbers camera and cloth to collect and ID fleas
	+ **Results:** no adverse effects (death, diarrhea) but flea abundance on PDs INCREASED from before to after treatment on all plots and was unchanged in burrows
* Part (III): n=15 PD, fed nitenpyram at different doses for 48 hrs, exposed to fleas for 24 hr, evaluated flea survival
	+ **Results:** flea survival declined with higher doses of nitenpyram but flea survival at 72 hr post-treatment was 80% suggesting very short half-life of nitenpyram

**KEY POINTS:**

* Lufenuron was maintained at detectable levels longer in torpid PDs than non-torpid PDs when given orally at a high dose (10x cat dose)
	+ Declined to <60 ppb at 7 weeks (torpid) and 3 weeks (non-torpid) respectively
* LNBits were not effective in controlling adult fleas on wild BTPD
* Most efficacious tools of flea control in PDs is deltamethrin dust and fipronil baits, both of which suppress for 12-24 months

**Terrestrial pathogen pollutant, *Toxoplasma gondii*, threatens Hawaiian monk seals (*Neomonachus schauinslandi*) following heavy runoff events.** *Journal of Wildlife Diseases*. 59.1. (2023): 1-11.

Laura Martinelli

**Abstract:** Toxoplasmosis is a major threat to Hawaiian monk seals (*Neomonachus schauinslandi*) in the main Hawaiian Islands where seal habitat overlaps with substantial human and domestic cat populations. **As the definitive hosts, members of the Felidae are the sole sources contaminating the environment with infectious oocysts; these oocysts can be transported into the marine environment, thereby threatening marine mammals.** To understand environmental factors influencing Hawaiian monk seal exposure to *Toxoplasma gondii*, **we examined monk seal strandings from toxoplasmosis in relationship to location and rainfall patterns throughout the main Hawaiian Islands.** Using a case-control study design, **we compared mortalities due to toxoplasmosis (cases) with those from other causes (controls).** We found that **cases were up to 25 times more likely than controls to occur after heavy runoff events.** The **greatest odds ratio was observed when rainfall occurred 3 wk before strandings**, potentially indicating important timelines in the disease process. **Our results suggest that heavy rainfall frequently delivers sufficient numbers of oocysts to infect Hawaiian monk seals. With infectious doses of as low as a single oocyst, any contaminated runoff constitutes a risk to Hawaii’s endangered monk seal.**

Key Points:

* Toxoplasma gondii can infect wide range of warm-blooded intermediate hosts, but can only complete sexual reproduction (producing oocysts) in definitive host Felidae
	+ Hawaii has no native felids, leaving domestic and feral cats as the only contributors of oocyst pathogen pollution
	+ Felidae can shed millions of oocysts in feces, leading to significant environmental contamination
	+ Oocysts infective >1 yr in soil, freshwater, or saltwater; Extremely hardy
* Toxoplasmosis (protozoan parasite) first identified as cause of death in wild Hawaiiam monk seal in 2004
	+ Cases observed on the main Hawaiian islands (with people and cats) and minimal cases have occurred on the NW Hawaiian islands (remote)
* Toxoplasmosis is leading cause of disease for H. monk seals (along with antrhopogenic trauma and net-related drownings)
* Elevated risk of stranding approximately 3 wks after exposure to heavy runoff, suggesting morbidity and mortality in infected Hawaiian monk seals develops relatively soon after exposure. (Note in humans, often latent or subclinical infections.)
* Hawaiian monk seals have large home ranges for foraging but small core use areas and tend to favor specific beaches (75% haul out time at a single region) so suspect Toxoplasma exposure likely occurs in this area and associated with level of oocyst contamination at that site

Take Home Point: Significantly elevated risk of toxoplasmosis in Hawaiian Monk Seals is associated with high runoff events in Hawaii.

LETHAL EFFECTS ON FLEA LARVAE OF FIPRONIL IN HOST FECES: POTENTIAL BENEFITS FOR PLAGUE MITIGATION

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ABSTRACT: Plague, caused by the bacterium Yersinia pestis, is a zoonotic disease of mammalian hosts and flea vectors. Fipronil baits have been used to suppress adult fleas for plague mitigation. The degree and duration of flea control may increase if fipronil also kills other stages in the flea life cycle. We fed grain treated with 0.005% fipronil by weight, or nontreated grain, to black-tailed prairie dogs (Cynomys ludovicianus), which excrete fipronil and metabolites in their feces after consuming fipronil in their diet. We presented prairie dog feces to 331 larval Oropsylla montana (Siphonaptera: Ceratophyllidae). When exposed to feces lacking fipronil or metabolites, 84% of larvae survived for 24h. In contrast, survival declined to 42% for larvae contacting feces from fipronil-treated prairie dogs. Just 7% of larvae consuming feces from fipronil-treated prairie dogs survived. Fipronil and metabolites may persist in host feces for several months or longer in prairie dog burrows where flea larvae dwell and forage. The lethal effects of fipronil on adult and larval fleas (and perhaps other life stages) may help to explain why fipronil baits are capable of suppressing fleas on prairie dogs for 12 mo.

* Yersinia pestis: One Health view needed- recognize human, animal and envionrmental health are linked
* Firponil: phenylpyrazole insecticide- can be applied by consumption of baits
	+ Chemicals block GABA(A) receptors and desensitize glutamate gated chloride channels= hyperexcitation, paralysis, and death
* Adult fleas only comprise up to 5% of population; whereas larva are >35% and are within rodent nests and feed on feces
* First experiment: 8% of larva consuming the fipronil residue in feces survived
* Second experiment: 88% survived that were exposed to control feces, 34% survived that contacted but did not consume the residue, and 7% survived that consumed the fipronil residue in feces
	+ No statistical difference between larvae that had consumed or not consumed BTPD feces.
* No evidence in this study that larva were trying to avoid the feces that had fipronil in it vs not
* Fipronil is light sensitive so in dark burrows could persist for 200 days or more= potential for long term flea control
* Treated baits have potential to expose all aspects of flea life cycle- but the egg stage may be protected and could potentially allow more flea eggs to survive and transition due to less larva that are alive to consume them
* Those that consume FipBits may also kill several flea life stages
* However, could continued treatment lead to resistance of fleas to fipronil and could accumulation in prairie dogs lead to toxicity in longer lived predators such as raptors

Browning, Geoffrey R., et al. "Outcomes of Transplacental transmission of *Toxoplasma Gondii* from chronically infected female red ruffed lemurs (*Varecia Rubra*)." *Journal of Zoo and Wildlife Medicine* 52.3 (2021): 1036-1041.

**Abstract:**

Ten red ruffed lemurs (*Varecia rubra*)—two adult females and their eight offspring—were evaluated in this case series. Two adult females were diagnosed with **chronic, latent toxoplasmosis** based on serologic testing. The first female lemur had two successive pregnancies. The first pregnancy resulted in **transplacental transmission of *Toxoplasma gondii****.* The only surviving **offspring** was diagnosed with **congenital toxoplasmosis** based on **serologic testing** and **compatible ophthalmic lesions**. The two **deceased offspring had disseminated nonsuppurative inflammation and intralesional protozoal organisms consistent with *T. gondii***, which was confirmed by polymerase chain reaction. **The second pregnancy did not result in transplacental transmission**. The second chronically infected adult female lemur had one pregnancy that resulted in a **single stillborn fetus without evidence of transplacental transmission of *T. gondii***. Treatment with trimethoprim-sulfamethoxazole and folinic acid was administered to the first adult female and one offspring, but no treatment was given to the second adult female. All surviving lemurs had no further complications associated with toxoplasmosis. This case series demonstrates that chronic, latent infection of reproductive female red ruffed lemurs with *T. gondii* may result in variable outcomes: (1) **transplacental transmission with disseminated fetal infection and stillbirth**, (2) **transplacental transmission with congenital infection and survival**, or (3) lack of transplacental transmission and **healthy offspring**. Information gained from these cases may help guide recommendations for breeding of this critically endangered species.

**Key Points:**

* *Toxoplasma gondii* is an apicomplexan protozoal parasite that infects an array of taxa. Felids = definitive hosts. Ingestion of sporulated oocysts or tissue cysts in intermediate hosts are common routes of transmission, but transplacental transmission can occur. In immunocompetent individuals, infection is often asymptomatic with chronic lifelong latency.
* Prosimians are very susceptible to toxoplasmosis, but clinical cases in red ruffed lemurs have not been reported. In ring-tailed lemurs (*Lemur catta*), acute disseminated toxoplasmosis has been reported. A ring-tailed lemur was also diagnosed with localized toxoplasmosis resulting in placentitis, stillbirths, and disseminated fetal infection.
* **Lemur A:** 6yo apparently healthy nulliparous female gave birth to triplets (A1a, A1b, A1c). *Toxoplasma gondii* was detected via PCR of one placenta and one deceased offspring, confirming in utero infection with *T. gondii* as the cause of death. Chronically elevated T. gondii IgG combined with multifocal inactive chorioretinitis supported a diagnosis of chronic toxoplasmosis. Therapy with TMS and folinic acid initiated to prevent reactivation and transplacental transmission during next pregnancy. One year later, gave birth to quadruplets. Two neonates found dead (lemurs A2a and A2b) and two neonates survived (lemurs A2c and A2d) with no evidence of toxoplasmosis.
* **Lemurs A1a, A1b:** On postmortem, both lemurs had nonsuppurative inflammation in multiple organs, including heart, brain, lung, adipose tissue, salivary gland, stomach, and testis. Protozoa were seen in lung, kidney, and adipose tissue. Nonsuppurative placentitis was present. PCR testing of placenta and liver was positive, with sequencing confirming *T. gondii*. Death in both was attributed to congenital toxoplasmosis.
* **Lemur A1c:** diagnosed with congenital toxoplasmosis based on serologic testing and compatible ophthalmic lesions. Treatment of humans with congenital toxoplasmosis improves long-term clinical outcomes; lemur A1c was placed on antiprotozoal therapy for 1 yr. At 5 yo, lemur A1c had no evidence of reactivation of latent toxoplasmosis.
* **Lemurs A2a and A2b:** Found dead. No evidence of toxoplasmosis in histologic sections, and PCR testing for *T. gondii* was negative for both animals.
* **Lemurs A2c and A2d:** Both survived. At 7 months Toxoplasma serology was negative.
* **Lemur B:** 10yo apparently healthy F. Serologic testing for *T. gondii* was positive. Singleton stillbirth. Transient lethargy but returned to normal mentation and activity.
* **Lemur B1a:** There were no gross or histopathologic findings of toxoplasmosis in the animal or placenta. Negative for *T. gondii* on PCR
* Lemur A demonstrates that a single episode of gestational reactivation of latent toxoplasmosis and transplacental transmission does not predict future gestational reactivation in a red ruffed lemur. Furthermore, reactivation of latent toxoplasmosis may not cause clinical disease in the dam, and transplacental transmission is not universally fatal to the offspring.
* Lemur B demonstrates that a red ruffed lemur with chronic, latent toxoplasmosis can have a first pregnancy free from reactivation and transplacental transmission.



**Take-Home Message:**

* This case series describes birth outcomes of two red ruffed lemurs with serologic evidence of chronic latent toxoplasmosis. This is the first report of reactivation of latent toxoplasmosis in an adult female red ruffed lemur and nonfatal congenital toxoplasmosis in a prosimian. Additionally, gestational reactivation of latent toxoplasmosis is not universal, neither across individuals nor across successive pregnancies in a single individual.
* Chronic toxoplasmosis in female red ruffed lemurs does not necessarily preclude breeding, but clinicians should be aware of potential for gestational reactivation and transplacental infection.