DOI: 10.7589/2015-08-212

Journal of Wildlife Diseases, 52(2), 2016, pp. 436–438

Toxoplasmosis May Lead to Road Kills of Persian Leopards (*Panthera pardus saxicolor*) in Golestan National Park, Iran

Somayeh Namroodi,[1,4] Alireza Gholami,[2] and Ehsan Shariat-Bahadori[3] [1]Department of Environmental Sciences, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources Sciences, Golestan, Gorgan 0094, Iran; [2]WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran, Tehran 0094, Iran; [3]University of Tarbyat Modarres, Tehran 0094, Iran; [4]Corresponding author (snamroodi2000@yahoo.com)

ABSTRACT: Three Persian leopards (*Panthera pardus saxicolor*) that died from car accidents in Golestan National Park, Iran, were tested for *Toxoplasma gondii* and rabies virus infection. Acute *T. gondii* infection was diagnosed in two Persian leopards; no rabies virus was detected. Acute toxoplasmosis may be a factor in Persian leopard road kills.

Building roads within wildlife habitats endangers wildlife by causing habitat loss and fragmentation, mortality via road kills, and declines in species' distribution and genetic diversity. The increased stress caused by these conditions can result in immunosuppression and increased susceptibility to a wide range of infectious diseases (Burnett 1992; Baskaran and Boominathan 2012; Hollings et al. 2013).

Golestan National Park (GNP) is a 92,000-ha area in northeastern Iran that provides protected natural habitat for 50% of Iran's total mammal species, including the Persian leopard (*Panthera pardus saxicolor*). The Persian leopard is an endangered subspecies included in the International Union for Conservation of Nature Red List, and its largest population (550–850 individuals) survives in Iran, especially in GNP (Kiabi et al. 2002; Nowell et al. 2008; Ziaie 2009).

Sanei et al. (2012) recorded 71 Persian leopard mortalities from 2007 to 2011 in Iran. The highest number of mortalities (n=16; 23% of the total) was at GNP. Eleven leopards were hunted by poachers and five were killed in road accidents; only one leopard died naturally.

The Asian Highway, which passes through the GNP, has a large negative impact on Persian leopard habitat and causes a high number of mortalities from collisions with vehicles. Vehicle collisions with animals can happen due to the high speed of the vehicles and blinding by headlights (Baskaran and Boominathan 2012). Nevertheless, it is also likely that Persian leopards suffer from infectious diseases such as acute toxoplasmosis and rabies that can lead to blindness or neurologic disorders, which would contribute to road kills (Dubey and Lappin 2006).

Hollings et al. (2013) found a higher prevalence of antibody to *Toxoplasma gondii* in road-killed feral cats than in culled individuals, suggesting there may be behavioral changes associated with toxoplasmosis leading to increased risk of road kills. This phenomenon may also occur in Persian leopards. Analysis of data collected about the diseases of road-killed leopards could provide insights into the causes and circumstances behind these incidents. *Toxoplasma gondii* is an obligate intracellular parasite that causes a wide range of symptoms such as uveitis, chorioretinitis, neurologic deficits, dermatitis, and death in its hosts (mammals and birds) from inflammatory processes (Dubey and Lappin 2006).

Despite the high number of Persian leopard road kills and the recognition of the Persian leopard as a keystone species, there are very few data about the principal causes of Persian leopard road kills and also about the epidemiology of their infectious diseases. The carcasses of three road-killed Persian leopards found along the Asian Highway in GNP were transferred to the laboratory during 2013–14. After dissection, transtracheal wash fluids, peritoneal, and pleural fluids were subjected to cytologic examination for *T. gondii* tachy-

zoites. To detect *T. gondii*–encysted bradyzoites, brain, liver, kidney, and lung tissues were isolated and placed in 10% neutral-buffered formalin (Sigma, Munich, Germany), processed for paraffin embedding, sectioned at 5 μm, and stained with H&E (Silva et al. 2008).

Fecal samples were tested via fecal flotation. Serum samples, obtained from the heart within several hours of death, were tested at twofold dilutions starting at 1:20, with formalin-fixed whole tachyzoites as antigen and the addition of 2-b-mercaptoethanol to detect immunoglobulin M (IgM) antibody to *T. gondii*. The cutoff titer (expressed as the reciprocal of the highest dilution positive) was 20 as recommended by Dubey et al. (1995).

Brain samples were tested for rabies virus by fluorescent antibody test. We made 15-mm–diameter smears from brain tissues and dried them completely at room temperature prior to fixation for 15–30 min. Slides were transferred to a cold acetone container and kept 1 hr at 20 C. Anti-rabies nucleocapsid fluorescein isothiocyanate–conjugated antibody was added to slides and the slides were incubated for 30 min at 37 C in a high-humidity chamber. After staining, and draining of excess conjugate, the slides were immersed in phosphate-buffered saline for 3–5 min. Microscopic observation of apple-green fluorescence was considered as an indicator of rabies antigens. Tachyzoites and encysted bradyzoites of *T. gondii* were observed in peritoneal fluid and brain tissue by H&E staining, respectively, in two Persian leopards. No *T. gondii* oocysts were detected in fecal samples. Fluorescent antibody tests for rabies virus were all negative. Other findings including sex and age of the leopards examined are provided in Table 1.

Positive IgM titers of anti-*T gondii* (80 and 160) and presence of *T. gondii* tachyzoites in peritoneal effusion (Table 1) indicate acute toxoplasmosis in two of the three surveyed Persian leopards (Dubey and Lappin 2006). Also, on postmortem examination hepatomegaly was observed in one Persian leopard that was positive for *T. gondii* IgM with a titer of 80.

TABLE 1. Description and clinical findings for three road-killed Persian leopards (*Panthera pardus saxicolor*) from Golestan National Park, Iran, tested for infection with *Toxoplasma gondii*.[a]

Variable	Sampled Persian leopard		
	1	2	3
Sex	Male	Female	Male
Age (yr)	3–5	5–7	3–5
Toxoplasma gondii IgM titer	1:80	1:60	–
Tachyzoites	+	+	–
Necropsy findings	Hepatomegaly	NSF	NSF

[a] IgM = immunoglobulin M; NSF = no abnormality observed; – = negative; + = positive.

Documentation of acute toxoplasmosis in wild felids is rare. The first report of elevated IgM in a wild felid actively infected with *T. gondii* was a cheetah cub (*Acinonyx jubatus*) in the United Arab Emirates (Lloyd and Stidworthy 2007). Related studies have demonstrated latent toxoplasmosis by detecting *T. gondii* immunoglobulin G (IgG) antibody in wild felids. High titers of IgG antibody to *T. gondii* have been recorded in wild felids such as snow leopards, Amur leopards, and a Persian leopard at a zoo in the midwestern US (Camps et al. 2008).

We diagnosed acute toxoplasmosis or reactivation of latent infection, which can have the same signs, in two road-killed Persian leopards. Nevertheless, whether this disease contributed to the car accidents by blinding, neurologic disturbance, or behavioral alteration is not clear; additional studies are needed. We hope that our data will serve as a baseline for future comparisons. There is a clear need to limit highway construction and avoid vehicular collisions with wildlife in protected parks such as GNP. Nevertheless, more studies on infectious diseases of the endangered Persian leopard are necessary.

LITERATURE CITED

Baskaran ND, Boominathan D. 2012. Road kill of animals by highway traffic in the tropical forests of Mudumalai Tiger Reserve, southern India. *J Threat Taxa* 2: 753–759.

Burnett S. 1992. Effects of a rainforest road on movements of small mammals: Mechanisms and implications. *Wildl Res* 19:95–104.

Camps S, Dubey JP, Saville WJ. 2008. Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoos in the Midwestern United States. *J Parasitol* 94:648–653.

Dubey JP, Lappin MR. 2006. Toxoplasmosis and neosporosis. In: *Infectious disease of dogs and cats,* 2nd Ed., Greene CE, editor. Elsevier Health Sciences, Amsterdam, Netherlands, pp. 754–768.

Dubey JP, Lappin MR, Thulliez P. 1995. Long term antibody responses of cats fed *Toxoplasma gondii* tissue cysts. *J Parasitol* 81:887e93.

Hollings T, Jones M, Mooney N, McCallum H. 2013. Wildlife disease ecology in changing landscapes: Mesopredator release and toxoplasmosis. *Int J Parasitol* 2:110–118.

Kiabi BH, Dareshouri BF, Ghaemi RA, Jahanshahi M. 2002. Population status of the Persian leopard (*Panthera pardus saxicolor* Pocock, 1927) in Iran. *Zool Middle East* 26:41–47.

Lloyd C, Stidworthy MF. 2007. Acute disseminated toxoplasmosis in a juvenile cheetah (*Acinonyx jubatus*). *J Zoo Wild Med* 38:475–478.

Nowell K, Breitenmoser-Wursten C, Breitenmoser U, Hoffmann M. 2008. *Panthera pardus saxicolor*. IUCN Red List of threatened species. http://www.iucnredlist.org/details/15961/0. Accessed November 2015.

Sanei A, Mousavi M, Mousivand M, Zakaria M. 2012. Assessment of the Persian leopard mortality rate in Iran. In: *Proceedings of the annual symposium on sustainability science and management*, 9–11 July, University of Malaysia, Terengganu, Malaysia, pp. 1458–1462, e-ISBN 978-967-5366-93-2.

Silva AF, Oliveira FCR, Leite JS, Mello MFV, Brandão FZ, Leite RI, Frazao-Texieira E, Lilenbaum W, Fonseca AB, Ferreira AM. 2008. Immunohistochemical identification of *Toxoplasma gondii* in tissues from modified agglutination test positive sheep. *Vet Parasitol* 191:347–352.

Ziaie H. 2009. [*A field guide to the mammals of Iran,* 2nd Ed.] Iran Wildlife Center, Tehran, Iran, 290 pp. Persian.

Received for publication 20 August 2015.
Accepted 7 December 2015.