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Detection of *Hepatozoon felis* in Ticks Collected from Free-Ranging Amur Tigers (*Panthera tigris altaica*), Russian Far East, 2002–12

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ABSTRACT: We collected 69 ticks from nine, free-ranging Amur tigers (*Panthera tigris altaica*) between 2002 and 2011 and investigated them for tick-borne pathogens. DNA was extracted using alkaline digestion and PCR was performed to detect apicomplexan organisms. Partial 18S rDNA amplification products were obtained from 14 ticks from four tigers, of which 13 yielded unambiguous nucleotide sequence data. Comparative sequence analysis revealed all 13 partial 18S rDNA sequences were most similar to those belonging to strains of *Hepatozoon felis* (>564/572 base-pair identity, >99% sequence similarity). Although this tick-borne protozoon pathogen has been detected in wild felids from many parts of the world, this is the first record from the Russian Far East.

Key words: Amur tiger, *Hepatozoon felis*, Russian Far East, tick-borne disease.

Amur tigers (*Panthera tigris altaica*) are found primarily in the Russian Far East (RFE). Approximately 80% of tiger deaths in the RFE are thought to be related to human activity (Goodrich et al. 2008; Robinson et al. 2015), but recent studies identified infectious disease as an emerging concern (Quigley et al. 2010; Gilbert et al. 2015). The impact of infectious disease can increase in small populations with decreased genetic diversity (Smith et al. 2009), such as that found in the remaining population of free-ranging Amur tigers (Russello et al. 2004). We investigated the presence of *Hepatozoon* in ticks collected from Amur tigers between 2002 and 2011 and discuss its potential effect on tiger conservation.

Ticks were collected from Amur tigers captured during a long-term research program (Goodrich et al. 2012) on the Sikhote-Alin Biosphere Zapovednik, Primorye Province, RFE (44°46′N, 135°48′E) and stored in 70% ethanol. We identified ticks to genus level by microscopic examination of morphologic features (Walker et al. 2014). We extracted DNA from individual ticks using alkaline digestion (Bown et al. 2003) and incorporated DNA extracts into a nested PCR assay to amplify an approximately 700-base pair (bp) fragment of the 18S rRNA-encoding gene (18S rDNA) of hemoparasitic members of the phylum Apicomplexa (including members of *Babesia* and *Hepatozoon*), as described by Simpson et al. (2005). Process blanks comprised of tubes containing 250 μL of 1.25% ammonia solution only were coprocessed with samples at a ratio of 1:5. The PCR products were resolved electrophoretically on 1.5% (w/v) agarose gels containing GelRed™ fluorescent nucleic acid stain (Biotium Inc., Hayward, California, USA) and were visualized under ultraviolet light. Amplicons were purified using the PureLink™ PCR Purification kit (Invitrogen, Life Technologies, Paisley, UK), and the nucleotide base sequences of both strands of a subset of these samples were determined commercially (Source Bioscience, Nottingham, UK) with the primers used in the final round of the nested PCR described earlier. Raw sequence data were verified and combined using ChromasPro (Technelsyium, South Brisbane, Queensland, Australia) and primer sequences at the extremities of each consensus sequence were removed. Comparative sequence analysis of the remaining sequence data was performed using BLAST (National Center for Biotechnology Information 2016).

We identified 69 ticks from nine tigers as belonging to the genera *Ixodes* (22 males, 13 of which were attached to females, and 27 females), *Haemaphysalis* (seven males and 12 females), or *Dermacentor* (one female). Of these 69 ticks, 14 (20%) from four animals yielded a PCR amplification product. Thirteen of these were *Ixodes* spp. (11 females, 2 males) and one was a female *Haemaphysalis* sp. Thirteen of the amplification products yielded unambiguous sequence data of at least 572 bp. No unambiguous differences were observed between the 13 sequences, and the consensus sequence shared 99% sequence similarity (564/572 bp) with an 18S rDNA fragment of *Hepatozoon felis* isolate LaCONES/Asiatic lion 02 (GenBank accession HQ829439). The next 32 best BLAST matches were partial 18S rDNA sequences derived from *H. felis* strains, and the amount of variation observed among these sequences (up to 2% sequence dissimilarity, up to 10 single nucleotide polymorphisms) was greater than that observed between our sequence and HQ829439. The most similar 18S rDNA sequence from a different taxon was a *Martes martes*-associated hepatozoon (GenBank EF222257) which shared 97% sequence similarity (556/572 bp). These observations provide evidence that ticks collected from Amur tigers contain DNA derived from strains of *H. felis*. Free-ranging Amur tigers are clearly exposed to *H. felis* and could act as a reservoir host. The detection of *H. felis* DNA in two (nonfeeding) male *Ixodes* ticks suggests that this species can maintain infection transstadially and thus may be a competent vector for the parasite.

Hepatozoon felis occurs in wild felids in many parts of the world including Africa (Williams et al. 2014), India (Pawar et al. 2012), Brazil (Andre et al. 2010), Thailand (Salakij et al. 2010), Japan (Sakuma et al. 2011), Iran (Khoshnegah et al. 2012), and Korea (Kubo et al. 2010). *Hepatozoon* spp. infections in wild carnivores generally are asymptomatic (Andre et al. 2010; Cunningham and Yabsley 2012; Pawar et al. 2012), and there are no reports of *Hepatozoon*-related disease in wild felids. Infection of immunocompetent domestic cats with *Hepatozoon* spp. does not usually result in morbidity, but clinical disease occurred in individuals coinfected with immunosuppressive viruses such as feline immunodeficiency virus or feline leukemia virus (Baneth and Vincent-Johnson 2005).

Mathematical modeling of tiger population growth and decline suggests that populations are sensitive to very small decreases in survival and reproduction (Chapron et al. 2008). Any pathogen with the potential to immunosuppress may increase susceptibility of Amur tigers to vector-borne pathogens such as *H. felis*, potentially leading to increased mortality. Previous studies demonstrated that free-ranging Amur tigers are exposed to a range of feline pathogens including feline corona virus, canine distemper virus, feline parvovirus, and *Toxoplasma gondii* (Goodrich et al. 2012). In addition, antibodies to feline immunodeficiency virus and feline-associated *Chlamydia* and *Mycoplasma* species were identified in feral cats in the RFE (Gonchuruk et al. 2012). Several of these disease agents have the potential to immunosuppress infected animals, as does the high inbreeding coefficient of the Amur tiger population (Smith et al. 2009).

Although it is unlikely that *H. felis* would cause clinical disease in healthy adult tigers, consideration should be given to potential detrimental effects of this pathogen on a declining population. Pathogen-associated population declines, such as those seen with canine distemper in African lions (*Panthera leo*; Roelke-Parker et al. 1996) and rabies in Ethiopian wolves (*Canis simensis*; Sillero-Zubiri et al. 1996), occur around the world. Identification of potential pathogens and the development of mitigation strategies to decrease their potential impact should be an important part of ongoing conservation efforts for wild tigers.

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LITERATURE CITED

André MR, Adania CH, Teixeira RHF, Vargas GH, Falcade M, Sousa L, Salles AR, Allegretti SM, Felippe PAN, Machadoet RZ. 2010. Molecular detection of *Hepatozoon* spp. in Brazilian and exotic wild carnivores. *Vet Parasitol* 173:134–138.

Baneth G, Vincent-Johnson N. 2005. Hepatozoonosis. In: *Arthropod-borne infectious diseases of the dog and cat*, Shaw SE, Day MJ, editors. Manson Publishing Ltd., London, UK, pp. 78–89.

Bown KJ, Begon M, Bennett M, Woldehiwet Z, Ogden NH. 2003. Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. *Emerg Infect Dis* 9:63–70.

Chapron G, Miquelle D, Lambert A, Goodrich J, Legendre S, Clobert S. 2008. The impact on tigers of poaching versus prey depletion. *J Appl Ecol* 45: 1667–1674.

Cunningham MW, Yabsley MJ. 2012. Primer on tick-borne diseases in exotic carnivores. In: *Fowlers zoo and wild animal medicine current therapy*, Vol. 7, Miller RE, Fowler ME, editors. Elsevier Saunders, St. Louis, Missouri, pp. 458–464.

Gilbert M, Soutyrina SV, Seryodkin IV, Sulikhan N, Uphyrkina OV, Goncharuk M, Matthews L, Cleaveland S, Miquelle DG. 2015. Canine distemper virus as a threat to wild tigers in Russia and across their range. *Integr Zool* 10:329–343.

Goncharuk MS, Kerley LL, Naidenko SV, Rozhnov VV. 2012. Prevalence of seropositivity to pathogens in small carnivores in adjacent areas of Lazovskii Reserve. *Zool Zh* 91:355–361.

Goodrich JM, Kerley LL, Smirnov EM, Miquelle DG, McDonald L, Quigley HB, Hornocker MG, McDonald T. 2008. Survival rates and causes of mortality of Amur tigers on and near the Sikhote-Alin Biosphere Zapovednik. *J Zool* 276:323–329.

Goodrich JM, Quigley KS, Lewis JCM, Astafiev AA, Slabi EV, Miquelle DG, Smirnov EN, Kerley LL, Armstrong DL, Quigley HB, et al. 2012. Serosurvey of free-ranging Amur tigers in the Russian Far East. *J Wildl Dis* 48:186–189.

Khoshnegah J, Mohri M, Mirshahi A, Mousavi SJ. 2012. Detection of *Hepatozoon* sp. in a Persian leopard (*Panthera pardus ciscaucasica*). *J Wildl Dis* 48:776–780.

Kubo M, Jeong A, Kim S, Kim Y, Lee H, Kimura J, Agatsuma T, Sakai H, Yanai T. 2010. The first report of *Hepatozoon* species infection in leopard cats (*Prionailurus bengalensis*) in Korea. *J Parasitol* 96: 437–439.

National Center for Biotechnology Information (NCBI). 2016. Basic Local Alignment Search Tool (BLAST). http://blast.ncbi.nlm.nih.gov/Blast.cgi. Accessed April 2016.

Pawar RM, Poornachandar A, Srinivas P, Rao KR, Lakshmikantan U, Shivaji S. 2012. Molecular characterisation of *Hepatozoon* spp. infection in endangered Indian wild felids and canids. *Vet Parasitol* 186: 475–479.

Quigley KS, Evermann JF, Leathers CW, Armstrong DL, Goodrich J, Duncan NM, Miquelle DG. 2010. Morbillivirus infection confirmed in a wild Siberian tiger in the Russian Far East. *J Wildl Dis* 42:1252–1256.

Robinson HS, Goodrich JM, Miquelle DG, Miller CS, Seryodkin IV. 2015. Mortality of Amur tigers: The more things change, the more they stay the same. *Integr Zool* 10:344–353.

Roelke-Parker ME, Munson L, Packer C, Kock R, Cleaveland S, Carpenter M, O'Brien SJ, Pospischil A, Hofmann-Lehmann R, Lutz H, et al. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379:441–445.

Russello MA, Gladyshev E, Miquelle D, Caccone A. 2004. Potential genetic consequences of a recent bottleneck in the Amur tiger of the Russian Far East. *Conserv Genet* 5:707–713.

Sakuma M, Nishio T, Nakanishi N, Izawa M, Asarai Y, Okamura M, Miyama TS, Setoguchi A, Endo Y. 2011. A case of Iriomote cat (*Prionailurus bengalensis iriomotensis*) with *Hepatozoon felis* parasitemia. *J Vet Med Sci* 73:1381–1384.

Salakij C, Sirinarumitr T, Tongthainun D. 2010 Molecular characterization of *Hepatozoon* species in a leopard cat (*Prionailurus bengalensis*) from Thailand. *Vet Clin Pathol* 39:199–202.

Sillero-Zubiri C, King AA, Macdonald DW. 1996. Rabies and mortality in Ethiopian wolves (*Canis simensis*). *J Wildl Dis* 32: 80–86.

Simpson VR, Panciera RJ, Hargreaves J, McGarry JW, Scholes SFE, Bown KJ, Birtles RJ. 2005. Myocarditis and myositis due to infection with *Hepatozoon* species in pine martens (*Martes martes*) in Scotland. *Vet Rec* 156:442–446.

Smith KF, Acevedo-Whitehouse K, Pedersen AB. 2009. The role of infectious diseases in biological conservation. *Anim Conserv* 12:1–12.

Walker AR, Bouattour A, Camicas J-L, Estrada-Pena A, Horak IG, Latif AA, Pegram RG, Preston, PM. 2014. *Ticks of domestic animals in Africa: A guide to identification of species*, 2nd Ed. Bioscience Reports, Edinburgh Scotland, UK, 221 pp.

Williams BM, Berentsen A, Shock BC, Teixiera M, Dunbar MR, Becker MS, Yabsley MJ. 2014. Prevalence and diversity of *Babesia*, *Hepatozoon*, *Ehrlichia*, and *Bartonella* in wild and domestic carnivores from Zambia, Africa. *Parasitol Res* 113:911–918.

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