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## Exposure of Wild Sumatran Tiger (*Panthera tigris sumatrae*) to Canine Distemper Virus

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**ABSTRACT:** Canine distemper virus (CDV) is recognized as a conservation threat to Amur tigers (*Panthera tigris altaica*) in Russia, but the risk to other subspecies remains unknown. We detected CDV neutralizing antibodies in nine of 21 wild-caught Sumatran tigers (42.9%), including one sampled on the day of capture, confirming exposure in the wild.

As populations of threatened species such as tigers (*Panthera tigris*) continue to decline and fragment, they become increasingly vulnerable to stochastic events such as infectious disease outbreaks. Since 2003, infection with canine distemper virus (CDV) has been responsible for mortality of endangered Amur tigers (*P. tigris altaica*) in Russia and is estimated to increase the 50-yr extinction probability of small populations of 25 individuals by as much as 65% (Seimon et al. 2013; Gilbert et al. 2014, 2015, 2020). There is an urgent need to evaluate the CDV infection status of other tiger subspecies, to determine the potential threat to these vulnerable populations. Testing strategies based on the detection of pathogens (such as reverse transcriptase-PCR) are more useful for syndromic surveillance, where infection is suggested by clinical presentation for agents such as CDV that are associated with acute disease and short periods of shedding. These approaches can lead to epidemiologically valuable information (such as sequence data) but are limited by the infrequency of sampling opportunities for what are liable to be rare infection events in a species that is seldom handled. Serological approaches can represent a more cost-effective strategy to detect

population exposure to pathogens such as CDV that evoke a strong and long-lasting humoral immune response in animals that recover from infection (Greene and Appel 2006; Brown et al. 2010). We sought to determine whether wild populations of Sumatran tigers (*P. tigris sumatrae*) have been exposed to CDV, as a first step toward determining the potential threat that the virus might represent to the subspecies.

Sumatran tigers are classified as critically endangered by the *International Union for the Conservation of Nature*, with fewer than 500 individuals thought to exist in the wild (Linkie et al. 2008). Remaining habitat is highly fragmented and, although tiger sign has been positively identified in 33 areas (Wibisono and Pusparini 2010), many populations are small, isolated, and vulnerable to stochastic processes such as infectious disease introductions.

Serum samples were collected from 21 Sumatran tigers captured from the wild between 1988 and 2016. All captures were carried out as part of routine wildlife management and conflict resolution, with the exception of three tigers injured by snares. All 21 tigers were transferred to the zoological collection Taman Safari Indonesia (Bogor, West Java), where samples were collected by park veterinarians. These samples were then stored at –80 C and heat inactivated at 56 C for 30 min prior to testing. Serum neutralization assays were conducted as described by Logan et al. (2016), using HEK293 cells expressing canine signaling lymphocytic activation molecule (SLAM-F1, the receptor used by CDV for cell entry) and a replication-

deficient vesicular stomatitis virus pseudotype expressing hemagglutinin and fusion surface glycoproteins from the Onderstepoort strain of CDV. The referenced protocol was adapted slightly by substituting green fluorescent protein for luciferase to indicate infection, which negated the need for a luminometer. All assays were run in quadruplicate using four-fold serial serum dilutions ranging from 1:16 to 1:16,384. Fluorescent cells were counted manually in each well, with antibody titers determined as the highest dilution with  $\geq 90\%$  reduction in infected cells compared to the average count from four serum-free control wells. Final titers were calculated using the Spearman-Kärber method (Lorenz and Bögel 1973), and a titer of 1:16 or higher was considered positive.

A total of nine samples were positive (42.9%, 95% confidence interval: 21.8–66.0%,  $n=21$ ) with titers ranging from 1:128 to  $>1:16,384$ . Of these, eight positive samples were collected between 6 mo and 20 yr after capture from the wild, preventing differentiation of wild exposures from infections possibly contracted while in captivity. None of the tigers showed clinical signs indicative of CDV infection on the day of capture. The longevity of circulating antibodies in tigers that have recovered from CDV infection is unknown, but in dogs, neutralizing antibodies remain detectable for many years and possibly the remainder of the animal's life (Bohm et al. 2004; Greene and Appel 2006). Although no cases of clinical disease consistent with CDV had been recorded in the facility where these eight positive tigers were held, all were housed in enclosures with the potential for access by wild civets or other susceptible wildlife, representing a possible route for captive infection. One adult male tiger that was rescued from a snare in Bengkulu province, was found to have a titer of 1:8,192 and had been sampled by Taman Safari Indonesia veterinarians on the day of capture (12 January 2012), providing clear evidence that exposure had occurred in the wild. This tiger subsequently died from his injuries, apparently unrelated to CDV. The eight other seropositive tigers originated from Aceh, North Sumatra, and Bengkulu provinces.

Our pilot study confirms the occurrence of infection, but is unable to assess extent of exposure, which would be critical to evaluate the threat that CDV represents for Sumatran tiger populations. This could be rectified by routinely analyzing serum samples collected whenever wild tigers are captured for conflict resolution, rehabilitation, or biological research. In Russia since 2000, CDV seroprevalence in Amur tiger populations has been measured as 37.0% (confidence interval: 24.6–51.3%,  $n=54$ , Gilbert et al. 2020), providing a source of comparison. Furthermore, field staff and wildlife managers should be familiarized with the clinical profile of CDV in tigers, principally neurological disease (unusual behavior, loss of fear, loss of aggression, blindness, ataxia, and/or seizures), and possibly respiratory and gastrointestinal signs. In these cases, diagnostic testing (reverse transcription polymerase chain reaction with sequencing confirmation) of antemortem samples (whole blood, serum, conjunctival swabs, and/or urine), and postmortem samples (including brain tissue, lymph nodes, lung, and bladder) should become routine (see Seimon et al. 2013 for protocols). Demonstration of supporting histopathology and possibly use of immunohistochemistry would also be valuable. Widespread exposure would indicate the need to develop population viability models integrating CDV epidemiology to explore the impact of infection scenarios and identify populations at particular risk. Where required, epidemiological research aimed at identifying CDV reservoirs for Sumatran tigers would be essential to inform appropriate mitigation strategies, including the potential for vaccination of reservoir species or tigers themselves.

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