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## Serologic Survey of Selected Pathogens in Free-ranging Bengal Tigers (*Panthera tigris tigris*) in Nepal

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**ABSTRACT:** Serum samples of 11 Bengal tigers (*Panthera tigris tigris*) from Chitwan National Park in Nepal, collected between 2011–17, were evaluated for the presence of antibodies to eight diseases commonly investigated in large felids. This initial serologic survey was done to establish baseline information to understand the exposure of Nepal's free-ranging tiger population to these diseases. Tiger serum samples collected opportunistically during encounters such as translocation, human conflict, and injury were placed in cold storage for later use. Frozen serum samples were assessed for feline coronavirus (FCoV), feline immunodeficiency virus, feline leukemia virus, feline herpesvirus (FHV), canine distemper virus, canine parvovirus-2 (CPV-2), leptospirosis (LEP; seven serovars), and toxoplasmosis (TOX). Six tigers were found to be positive for LEP, eight for CPV-2, five for FHV, one for FCoV, and 10 for TOX. Tigers, like other wild felids, have been exposed to these common pathogens, but further research is needed to determine the significance of these pathogens to the Nepali population.

**Key words:** Bengal tiger, canine parvovirus, feline coronavirus, feline herpes virus, leptospirosis, Nepal, *Panthera tigris tigris*, toxoplasma.

Asia's tiger (*Panthera tigris*) populations are in decline across their range. As of April 2016, the global wild tiger population estimation was around 3,890, occupying about 7% of its historical range, mostly found in small pockets with <25 animals (Walston et al. 2010). Threats to tigers include habitat loss or fragmentation, decrease in prey density, wildlife trade and poaching, human-wildlife conflict, and infectious diseases (Dinerstein et al. 2007). Infectious disease is recognized as an increasing concern for wild carnivore populations globally, contributing to signifi-

cant declines. Canine distemper virus (CDV) in African wild dogs (*Lycaon pictus*) and lions (*Panthera leo*) as well as feline leukemia virus (FeLV) in Iberian lynx (*Lynx pardinus*) has led to several of these declines (Roelke-Parker et al. 1996; Meli et al. 2009; Woodroffe et al. 2012).

In Nepal, the tiger population has been growing, with the most recent report of 235 individuals, an increase of 19% since 2013 (National Trust for Nature Conservation 2018). Little is known about the diseases that affect tigers in Nepal. Because there has been recent evidence of diseases such as canine distemper in tigers in other areas of Asia and Russia, it is important to determine which infectious diseases may threaten the Nepali population to minimize the potential impacts on population recovery (Goodrich et al. 2012; Gilbert et al. 2015). Using serology, we determined baseline exposure to pathogens in Bengal tigers (*Panthera tigris tigris*) in Chitwan National Park including feline coronavirus (FCoV), feline immunodeficiency virus (FIV), FeLV, feline herpesvirus (FHV), CDV, canine parvovirus-2 (CPV-2), leptospirosis (LEP; seven serovars), and toxoplasmosis (TOX).

The study was conducted in Chitwan National Park in the Terai region of south-central Nepal (Fig. 1). Blood samples from 11 adult tigers were collected by opportunistic sampling from 2011–17 when tigers were captured, translocated, or held in captivity for various injuries or illnesses. Many were human-conflict tigers and were captured near human settlements. Captures were led by the

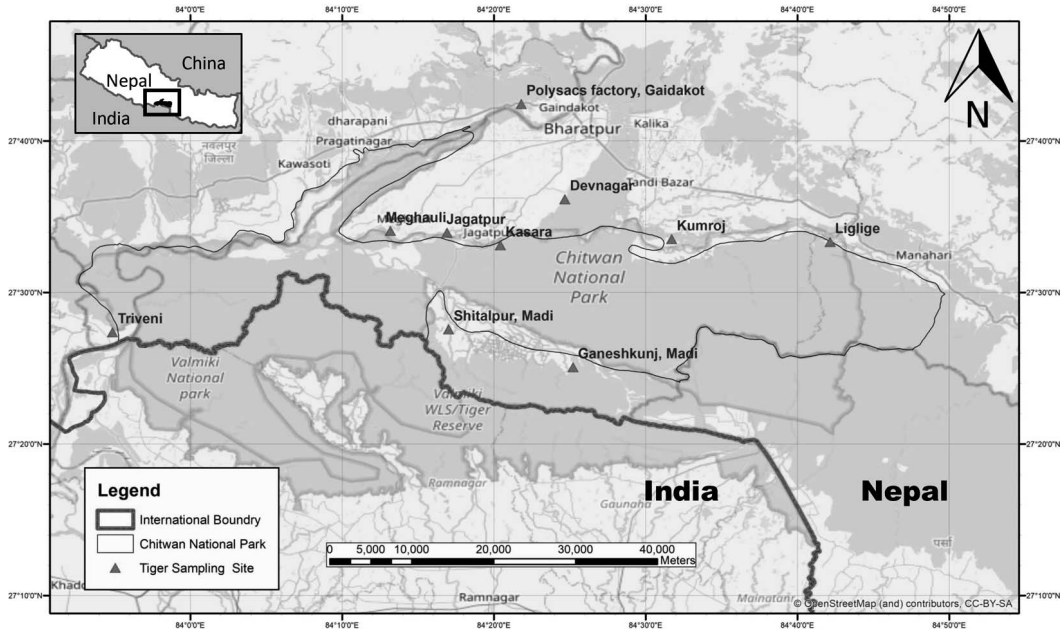


FIGURE 1. The study area includes Chitwan National Park and surrounding regions in south-central Nepal where a serologic survey of selected pathogens in Bengal tigers (*Panthera tigris tigris*) was done. Triangles represent locations of tiger captures and sampling during 2011–17.

Nepal Department of National Parks and Wildlife Conservation and National Trust for Nature Conservation. The tigers were captured by remote injection, anesthetized according to published protocol, and aged by tooth eruption and wear (Kreeger and Arneimo 2012). Blood samples were collected and the serum was extracted by centrifugation and stored in a  $-20\text{ C}$  freezer at the National Trust for Nature Conservation, Biodiversity Conservation Center in Sauraha, Nepal.

After obtaining US (no. 16US75654B/9) and Nepal (no. 1479876) Convention on International Trade in Endangered Species of Wild Fauna and Flora permits, serum samples were transported and evaluated at the New York State Diagnostic Laboratory at Cornell University in Ithaca, New York. Serum samples were evaluated using enzyme-linked immunosorbent assay to detect antibodies to FCoV, FIV, and TOX, and antigens to FeLV (Schaefer et al. 2011); serum neutralization to detect antibodies to FHV and CDV (Sheldon et al. 2017); hemagglutination inhibition to detect anti-

bodies to CPV-2 (Carmichael et al. 1980); and a microscopic agglutination test to detect antibodies to Pomona, Hardjo, Ictero, Grip-po, Canicola, Autumnalis, and Bratislava serovars of *Leptospira interrogans* (Cole et al. 1973).

Five of eight of the pathogens that we tested for had seropositive results and all but one of the tigers were seropositive for at least one of the pathogens (Table 1). All of the tigers were adults and therefore could not be compared based on their age. Seventy-three percent (8/11) of tigers tested positive for CPV-2 antibodies (with a titer  $\geq 80$ ), 45% (5/11) for FHV antibodies (with a titer of  $\geq 8$ ), 9% (1/11) for FCoV antibodies (with a titer  $\geq 1.20$ ), 91% (10/11) for *Toxoplasma gondii* antibodies (with a titer  $\geq 1.20$ ), and 55% (6/11) positive for LEP antibodies, including all serovars (with a titer  $> 100$ ). The highest serovar prevalences were Autumnalis with 36% positive, Hardjo with 27% positive, and Ictero with 18% positive. All samples were seronegative for FIV, FeLV, and CDV.

TABLE 1. Diagnostic summary of the serologic survey of selected pathogens in 11 adult Bengal tigers (*Panthera tigris tigris*) surveyed in and around Chitwan National Park, Nepal from 2011–17.

| Pathogen                      | No. seropositive tigers (n=11) |              |                  |            |
|-------------------------------|--------------------------------|--------------|------------------|------------|
|                               | Male (n=9)                     | Female (n=2) | Total n positive | % Positive |
| Feline coronavirus            | 0                              | 1            | 1                | 9          |
| Feline immunodeficiency virus | 0 <sup>a</sup>                 | 0            | 0                | 0          |
| Feline leukemia virus         | 0                              | 0            | 0                | 0          |
| Feline herpesvirus            | 3                              | 2            | 5                | 45         |
| Canine distemper virus        | 0                              | 0            | 0                | 0          |
| Canine parvovirus             | 6                              | 2            | 8                | 73         |
| Leptospira total              | 5                              | 1            | 6                | 55         |
| Leptospira Pomona             | 1                              | 0            | 1                | 9          |
| Leptospira Hardjo             | 3                              | 0            | 3                | 27         |
| Leptospira Ictero             | 2                              | 0            | 2                | 18         |
| Leptospira Grippo             | 0                              | 0            | 0                | 0          |
| Leptospira Canicola           | 1                              | 0            | 1                | 9          |
| Leptospira Autumnalis         | 3                              | 1            | 4                | 36         |
| Leptospira Bratislava         | 1                              | 0            | 1                | 9          |
| Toxoplasmosis                 | 8                              | 2            | 10               | 91         |

<sup>a</sup> One animal gave an equivocal positive result = suspected positive but not confirmed.

Canine parvovirus-2 antibody prevalence was high in sampled tigers. Canine parvovirus is closely related antigenically to feline panleukopenia virus (Ikeda et al. 2002). Canine parvovirus easily spreads in the environment, causing high morbidity and mortality with clinical signs including enteritis, depression, dehydration, and anorexia. A study on leopard cats (*Prionailurus bengalensis*) in Vietnam and Taiwan found that the viral isolates were more closely related to CPV in dogs than in domestic cats; therefore, it is more likely originating from dogs (*Canis lupus familiaris*) or wild carnivores than from domestic cats (*Felis catus*; Ikeda et al. 2002). The presence of a large, free-roaming domestic dog population in Chitwan, Nepal, along with wild carnivore populations in the park, could explain the high prevalence in tigers (Massei et al. 2017). A phylogenetic analysis of the virus in tigers, domestic dogs, and wild carnivores is needed to confirm this hypothesis in Nepal.

About one third of the tigers in this study were seropositive for FHV. The most common clinical signs in large felids are upper respiratory symptoms and rhinotracheitis, which

have been described in captive felids including cheetahs (*Acinonyx jubatus*), mountain lions (*Puma concolor*), jaguars (*Panthera onca*), and leopards (*Panthera pardus*; Hofmann-Lehmann et al. 1996). In Nepal, the origin and reservoir of FHV is unknown and further research is needed to determine how the tigers were exposed. This virus may be endemic in the population and may not cause many clinical signs or mortalities, as suggested with a study of lions in East Africa (Hofmann-Lehmann et al. 1996).

Feline coronavirus occurs in domestic and wild felids around the world. Feline coronavirus can induce feline infectious peritonitis or can cause mild clinical signs of enteritis (Hofmann-Lehmann et al. 1996). One tiger in this study was seropositive for FCoV. The tiger was captured from a community forest, was geriatric, severely dehydrated, weak, and cachectic. The clinical signs exhibited by this female could possibly be due to FCoV, as the titer was elevated, but this tiger was also seropositive for CPV, FHV and TOX, and coinfections could have contributed to morbidity (Table 2).

TABLE 2. A summary of coinfections of selected pathogens and reasons for capture of 11 adult Bengal tigers (*Panthera tigris tigris*) opportunistically sampled in Chitwan National Park and surrounding areas, Nepal from 2011–17.

| Tiger no. | Sample year | Sex    | Reason for capture                  | Coinfections <sup>a</sup> |
|-----------|-------------|--------|-------------------------------------|---------------------------|
| 1         | 2011        | Male   | Human conflict                      | LEP, TOX                  |
| 2         | 2011        | Male   | Livestock predation, injury         | FHV, CPV, LEP, TOX        |
| 3         | 2012        | Male   | Injury                              | LEP, TOX                  |
| 4         | 2012        | Male   | Human conflict                      | CPV, LEP, TOX             |
| 5         | 2013        | Female | Human conflict                      | FCoV, FHV, CPV, TOX       |
| 6         | 2013        | Male   | Predation                           | FHV, CPV, TOX             |
| 7         | 2013        | Male   | Livestock predation                 | FHV, CPV, LEP, TOX        |
| 8         | 2014        | Male   | Livestock predation, human conflict | CPV, TOX                  |
| 9         | 2015        | Male   | Unknown                             | Negative                  |
| 10        | 2016        | Male   | Unknown                             | CPV, TOX                  |
| 11        | 2017        | Female | Unknown                             | FHV, CPV, LEP, TOX        |

<sup>a</sup> LEP = leptospirosis (at least one of seven serovars); TOX = toxoplasmosis; FHV = feline herpesvirus; CPV = canine parvovirus-2; FCoV = feline coronavirus.

Almost all the tigers in this study were seropositive for TOX. *Toxoplasma gondii* is an intracellular protozoan that can infect humans and livestock, although the definitive host is domestic and wild felids (Furtado et al. 2015). Toxoplasmosis, transmitted via the fecal-oral route, is prevalent worldwide, is zoonotic, and can cause a variety of serious diseases in humans, livestock, and other wildlife (Tenter et al. 2000). *Toxoplasma gondii* is present wherever felids live, usually does not cause clinical signs, and prevalence can be up to 98% in a population (Spencer et al. 2003). Our results are consistent with other studies performed on wild felids (Goodrich et al. 2012; Furtado et al. 2015). This suggests that *T. gondii* is endemic in this population, most likely not causing clinical signs of disease but contributing to the maintenance of the pathogen in the study area.

About half of the tigers tested in this study were seropositive for at least one of the LEP serovars. *Leptospira* are Gram-negative bacteria, with small mammals considered the main reservoir, and are found most commonly in tropical areas that experience frequent flooding and high rainfall (Gamage et al. 2012). Leptospirosis is common in Nepal in domestic animals, humans, and wildlife and can be transmitted between them through

indirect or direct contact of urine (Shrestha et al. 2018). Known serovars found in humans in Nepal are Bratislava, Icterohaemorrhagiae, Autumnalis, and Sejroe (Gamage et al. 2012). One study on the *Leptospira* serovar Hardjo in large ruminants in Nepal revealed that prevalence in large ruminants in Nepal was 6.88% after the monsoon (Khanal et al. 2018). In our study of tigers, the most common serovars were Autumnalis, Hardjo, and Icterohaemorrhagiae. These serovars are important in human and livestock health, with human-livestock-wildlife interaction most likely playing a role in prevalence. It is unclear if LEP causes significant clinical disease in tigers, but exposure rates are high, most likely due to high small mammal populations, livestock predation, seasonal rain and flooding, and contaminated waterways.

Our baseline study revealed exposure of tigers to several important pathogens in Nepal, some of which are shared with humans and domestic animals. Further research is needed to determine the significance and origins of these pathogens. Although there is no known exposure to CDV, FIV, and FeLV, it is important to continue surveillance for these pathogens. It is possible that these viruses were not detected due to the small sample size available for the study. If the

population is truly naïve, then CDV and other pathogens could cause significant mortalities in the future as in other populations (Roelke-Parker et al. 1996; Meli et al. 2009; Woodroffe et al. 2012). Continued health surveillance of the tiger population in Nepal, including routine serology testing and necropsy information, is needed to support the growth of endangered populations and to develop policies to minimize morbidity and mortality from these diseases in the future.

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