NOTE *Wildlife Science*

Comparison of cortisol and thyroid hormones between tuberculosis-suspect and healthy elephants of Nepal

Sarad PAUDEL[1)#], Janine L. BROWN[2)#], Sharada THAPALIYA[3)], Ishwari P. DHAKAL[3)], Susan K. MIKOTA[4)], Kamal P. GAIRHE[5)], Michito SHIMOZURU[1)] and Toshio TSUBOTA[1)*]

[1)]*Laboratory of Wildlife Biology and Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060–0818, Japan*
[2)]*Smithsonian Conservation Biology Institute, Center for Species Survival, Front Royal, VA, U.S.A.*
[3)]*Agriculture and Forestry University, Rampur, Chitwan, Nepal*
[4)]*Elephant Care International, 166 Limo View Lane, Hohenwald, TN 38462, U.S.A.*
[5)]*Department of National Parks and Wildlife Conservation, Kathmandu, Nepal*

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ABSTRACT. We compared cortisol and thyroid hormone (T3 and T4) concentrations between tuberculosis (TB)-suspected (n=10) and healthy (n=10) elephants of Nepal. Whole blood was collected from captive elephants throughout Nepal, and TB testing was performed using the ElephantTB STAT-PAK® and DPP VetTB® serological assays that detect antibodies against *Mycobacterium tuberculosis* and *M. bovis* in elephant serum. Cortisol, T3 and T4 were quantified by competitive enzyme immunoassays, and the results showed no significant differences in hormone concentrations between TB-suspect and healthy elephants. These preliminary data suggest neither adrenal nor thyroid function is altered by TB disease status. However, more elephants, including those positively diagnosed for TB by trunk wash cultures, need to be evaluated over time to confirm results.

KEY WORDS: Asian elephant, cortisol, thyroxine, triiodothyronine, tuberculosis

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Tuberculosis (TB) in elephants is a re-emerging disease caused mainly by *Mycobacterium tuberculosis*, although infection by *M. bovis* also has been reported in a few cases [13, 14, 18]. The majority of elephants infected with TB do not show clinical symptoms, although in the advanced stage of disease, some will exhibit anorexia, weight loss, weakness and exercise intolerance. Bacterial culture of trunk wash samples is considered the gold standard for positive diagnosis; however, this technique is limited by poor sensitivity and only detects TB in animals that are actively shedding organisms which occurs intermittently and may not occur until late in the course of disease [11]. It also is a labor-intensive, time-consuming and costly procedure. Recently, two serological tests, the ElephantTB STAT-PAK® and DPP VetTB® (ChemBio Diagnostic Systems, Inc., Medford, NY, U.S.A.) that detect antibodies to *M. tuberculosis* and *M. bovis*, have been licensed by the United States Department of Agriculture (USDA) to screen for TB in elephants and have been found to be particularly useful for early detection [8, 10, 11].

In humans, biological responses to *M. tuberculosis* infection are involved in both the immunology and pathology of the disease. Cytokines released during the immune response in patients with TB activate the hypothalamo-pituitary-adrenal axis, which leads to the production of glucocorticoids [2] that can have anti-inflammatory actions [7]. Increased circulating cortisol has been observed in some patients with TB [2, 3], but no studies have examined if similar adrenal changes occur in TB-infected elephants.

Thyroid hormones include triiodothyronine (T3) and the active form thyroxine (T4) [7], and they play major roles in cellular differentiation, growth and metabolism, with their main effects on oxygen consumption and metabolic rates [22]. They are also involved in protein metabolism. One of the later stage signs of TB in elephants is weight loss. Thus, because TB is a wasting disease, it is plausible that altered thyroid function is associated with infection. Elevated thyroid hormone concentration has been reported in patients with TB [2], however, no studies have been conducted in elephants.

There are approximately 240 captive elephants managed under human care in Nepal and used in wildlife management and tourism. On-going surveillance using the DPP VetTB® test suggests ~25% of the population is infected with TB (unpublished), highlighting the seriousness of this problem in Nepal and perhaps other captive elephant populations. Captive elephants often interact with wild counterparts as well as other endangered wildlife species, such as the greater-one horned rhinoceros, and the opportunity therefore exists for the transmission of TB from infected elephants to other susceptible hosts. Thus, the monitoring of TB in captive elephants is an important component of captive management and health assessment. The purpose of this study was

*CORRESPONDENCE TO: TSUBOTA, T., Laboratory of Wildlife Biology and Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18 Nishi 9, Sapporo, Hokkaido 060–0818, Japan. e-mail: tsubota@vetmed.hokudai.ac.jp

#These authors contributed equally to this work.

to measure concentrations of serum cortisol, T3 and T4 in TB-suspects and healthy elephants to determine if adrenal or thyroid functions are altered by this disease.

The present study was conducted using 20 captive female elephants in five protected areas of Nepal: Chitwan National Park (n=13), Koshi Tappu Wildlife Reserve (n=2), Parsa Wildlife Reserve (n=2), Bardia National Park (n=2) and Shuklaphanta Wildlife Reserve (n=1).The mean age was 42.7 ± 2.41 (range, 15–64) years. Twelve elephants were owned by the government, and eight were privately owned. The government elephants were primarily used for patrolling protected areas for wildlife management and conservation purposes. The privately owned elephants were mainly used for tourist safaris in the national parks and the buffer zone community forests. Most of the elephants were housed in open-air, roofed stables adjoining other elephant stables. Elephants at each facility foraged and worked together. Every captive elephant was cared for by three handlers. Elephants were fed approximately 15 kg of rice, 2 kg of molasses and 500 g salt along with grasses and other supplements daily. The permission to carry out this research was obtained from the Department of National Parks and Wildlife Conservation (DNPWC), Ministry of Forests and Soil Conservation, Government of Nepal.

Blood was collected from a caudal auricular vein using 19-gauge winged IV infusion sets (Jor-Vet, Jorgensen Labs, Loveland, CO 80538) and adapters (Becton-Dickinson and Co., Franklin Lakes, NJ, U.S.A.) directly into 12.5 m*l* Corvac serum separator tubes (Kendall, Covidien, Mansfield, MA, U.S.A.). Blood was centrifuged within 6 hr at 1,500 ×g, and serum was stored in 2-m*l* cryovials (Fisher Scientific, Pittsburg, PA, U.S.A.). One vial from each elephant was used for immediate TB testing, and the remaining vial was stored at −20°C pending hormone analysis.

The ElephantTB STAT-PAK® and DPP VetTB® assays were performed according to the manufacturer's instructions; with results obtained within 20–30 min. Samples were first tested using the ElephantTB STAT-PAK®. The DPP VetTB® test was performed on samples with reactive results. This approach was used, because the STAT-PAK is highly specific and samples that are non-reactive on the STAT-PAK are unlikely to be reactive on the DPP. The DPP has demonstrated 100% sensitivity and specificity, and the STAT-PAK has shown 100% sensitivity and 95.2% specificity [8]. Elephants reactive on the DPP test were considered TB-suspects in this study as none of the elephants were positive on trunk wash culture during the sample collection period.

Hormone concentrations were determined using competitive enzyme immunoassays (EIA) (DiaMetra, Foligno, Italy) for cortisol (DKO001), free T3 (DKO037) and free T4 (DKO038) according to manufacturer's guidelines. Assay sensitivities for cortisol, free T3 and free T4 were 2.44 *n*g/m*l*, 0.05 *p*g/m*l* and 0.05 *n*g/d*l*, respectively. These kits were developed for clinical diagnostic use in humans, similar to those used in previous elephant studies of adrenal and thyroid functions [5, 6, 20]. Differences between TB-suspect and healthy control groups were determined using a non-parametric Mann-Whitney Test, and data are presented as mean ± SE.

Table 1. Mean (± SEM) and range of cortisol, T3 and T4 in TB suspected and healthy elephants

Hormones	TB-suspects	Healthy
Cortisol (*n*g/m*l*)	38.07 ± 9.83 (2.4–91.4)	34.72 ± 9.79 (2.4–77)
Free T3 (*p*g/m*l*)	2.69 ± 0.34 (1–4)	3.03 ± 0.25 (1.6–3.9)
Free T4 (*n*g/d*l*)	0.69 ± 0.05 (0.5–0.9)	0.67 ± 0.04 (0.4–0.8)

Of the 20 elephants sampled, 10 were reactive on both the STAT-PAK and DPP tests (43.3 ± 3.07 years of age), and 10 were non-reactive on the STAT-PAK (healthy controls; 42.1 ± 3.85 years of age). None of the elephants showed clinical signs of TB during the sampling period.

There was considerable variation in serum cortisol concentrations across individuals in both groups of elephants, but overall means were not significantly different between TB-suspects and healthy elephants ($P>0.05$) (Table 1). Similarly, there were no significant differences in concentrations of free T3 or T4 between TB-suspect and healthy elephants (Table 1).

This is the first study to compare serum cortisol, free T3 and free T4 hormone concentrations between TB-suspect and healthy elephants in Nepal where TB has been confirmed in the captive population [17]. Although there was considerable variation in hormone concentrations, there were no significant differences in adrenal or thyroid hormones between the groups. Active TB in human subjects has been associated with increased plasma levels of glucocorticoids [3], but this was not the case for these elephants. In fact, many TB-suspected elephants exhibited low levels of cortisol, so a hypo-adrenal problem cannot be ruled out.

Cortisol secretion in elephants is diurnal, with higher levels in the early morning that decline throughout the day before increasing after midnight [4]. Blood was collected from all the elephants in the morning, as this was the most suitable time based on their daily schedule. Studies in the U.S. report overall mean cortisol concentrations of ~25 *n*g/m*l*, ranging from 2.5 −74 *n*g/m*l*, for male and female Asian elephants [5, 6], although time of collection was not reported. Our slightly higher concentrations might be related to sample collection time, but could also be related to the different assays that were used. Those studies utilized a cortisol radioimmunoassay (RIA), which was found to detect lower concentrations when compared to an enzyme immunoassay (EIA) on elephant urine samples [4]. Thus, we cannot exclude sample matrix effects. Husbandry and management also were notably different between the zoo and Nepal populations, which could account for cortisol concentration differences. Elephants in this study were located in different places within the national parks, and the nature of their work also varied; i.e., patrolling and interacting with tourists at safari camps. Because of the greater variability in cortisol values in the TB-suspect group, it would be worth examin-

ing more animals, especially those with positive trunk wash cultures.

In human patients with pulmonary TB, T3 values have been found to be lower; although T4 levels were less affected [21]. In another study, there was a significant increase in thyroid hormone concentrations in TB patients [2]. However, we found no differences in thyroid function between TB-suspected and healthy elephants. Comparatively, mean values of 1.93 ± 0.26 *p*g/m*l* and 1.01 ± 0.06 *n*g/d*l* for free T3 and T4 were reported in healthy female Asian elephants [6], with concentrations of 1.72 ± 0.18 *p*g/m*l* and 0.72 ± 0.09 *n*g/d*l* in Asian bulls [5], respectively. In our study, T3 was slightly lower and T4 slightly higher compared to elephants in U.S. zoos. The differences in thyroid hormone concentrations among captive elephants in Nepal may have been due to geographical location, the nature of the work they performed or the different assay system (EIA vs RIA) used in our study. Overall, thyroid hormones did not appear to be altered by TB status.

Culture of trunk wash samples is considered a gold standard for diagnosis of TB in elephants [19]. Of the 20 study animals, 13 were located in Chitwan National Park or the adjacent buffer zone. Trunk wash samples were collected at least twice from these elephants and once from the two elephants that resided in Bardia National Park. Trunk washes were performed at least one month before blood collection. None of the cultures were positive. Failure to isolate TB bacteria does not rule out infection, because of the lower sensitivity of this test in elephants [10, 11]. In addition to reasons stated above, the bacterial load must have at least 100 colony forming-units per m*l* of specimen to be detected by culture [12]. Elephants must also be well trained for this procedure, and a poor sample can contribute to false negative readings. Other methods for diagnosing TB have proven impractical or unreliable in elephants. The intradermal tuberculin test is not accurate, and radiography (chest X-rays) is not feasible [9, 15].

Several studies have demonstrated the limitations of trunk wash culture for diagnosing TB. In Thailand, *M. tuberculosis* was isolated from only two of 60 trunk wash samples from three elephants that were confirmed TB positive on culture of necropsy samples [1]. In Europe, only 7 of 189 trunk wash samples were positive from five elephants confirmed by culture at post-mortem to be infected with TB [16]. By contrast, two serological tests, the DPP VetTB Assay and the Elephant TB STAT-PAK, have been licensed by the USDA as a screening test for TB in elephants. The STAT-PAK has since been discontinued, leaving the DPP VetTB Assay as the best serological test available, and it has been shown to have a high predictive value for the detection of TB infection in the elephants months or even years before *M. tuberculosis* can actually be isolated from trunk wash samples or at necropsy [10]. Elephants are unique among the TB susceptible host species in that they develop unusually robust antibody responses to the TB antigens. The sensitivity and specificity of the DPP assay have been shown to be 100% [8], and thus, this test appears to be superior for early diagnosis, in that antibody responses can be detected in infected elephants months to years prior to detection by culture [10, 11]. Although the stage of TB could not be determined in our study group, the reactive results on the DPP test strongly suggest they were infected. Differing stages of TB among the TB-suspect elephants might be contributing factors for the variation in hormone concentrations observed.

In conclusion, this study was the first to compare levels of cortisol, T3 and T4 in TB-suspect and healthy elephants, and found no apparent differences. Further studies are needed to assess longitudinal patterns of these hormones in a larger number of culture-confirmed and healthy elephants to determine if TB affects adrenal and thyroid functions during different stages of disease or with varying degrees of debilitation.

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