



Tuberculosis seroprevalence and comparison of hematology and biochemistry parameters between seropositive and seronegative captive Asian elephants of Nepal

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ABSTRACT. We conducted a tuberculosis (TB) serosurveillance program of captive elephants in Nepal and compared hematology and biochemistry parameters between seropositive and seronegative elephants. A total of 153 elephants (male=20, female=133) from four national parks were tested for TB using the ElephantTB STAT-PAK[®] Assay (ChemBio Diagnostic Systems, Inc., Medford, NY, USA). The mean reported age for 138 elephants was 38.5 years (range 2–71 years). Seroprevalence for TB was 21.56% (33/153). The majority of seropositive elephants were female (n=30) and from Chitwan National Park (n=29). The occurrence of TB seropositive cases in other more remote national parks suggests TB may be widespread among the captive elephant population of Nepal. Hematology and biochemistry analyses were performed on 13 and 22 seropositive elephants, respectively and, nine elephants from a seronegative TB herd for comparison. Hematology parameters (hemoglobin, packed cell volume, platelet, white blood cells, and erythrocyte sedimentation rate) were comparable between the two groups. Total protein, globulin, and lactate dehydrogenase were significantly higher in seronegative elephants, and bilirubin was significantly higher in seropositive elephants whereas blood urea nitrogen, creatinine, glutamic oxaloacetic transaminase/aspartate aminotransferase (GOT/AST), glutamic pyruvic transaminase/alanine aminotransferase (GPT/ALT), gamma glutamyl transferase (GT), and albumin were not significantly different. The range of biochemical parameters that were significantly different between seropositive and seronegative elephants had narrow ranges. Thus, the potential of these parameters as a direct biomarker for TB diagnosis is limited based on the findings in this study. We recommend including blood parameters in future TB surveillance studies.

KEY WORDS: biochemistry, elephant, hematology, seroprevalence, tuberculosis

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Tuberculosis (TB) has been recognized as an important health problem of captive Asian elephants (*Elephas maximus*). Asian elephants are mainly reported to be affected and the majority of cases are due to *Mycobacterium tuberculosis*, a causative agent of human TB [19, 22]. Culture-confirmed cases and TB seroprevalence rates in captive elephants have been reported from several Asian elephant range countries: Nepal [20, 25–27], Thailand [2–3, 35], India [36], Malaysia [24], and Laos [12]. TB has also been reported in captive elephants from USA [22, 23], Australia [37], Sweden [13], and Japan [39], suggesting a worldwide distribution. *M. tuberculosis* has been isolated from dead wild Asian elephants in India [5, 40] and Sri Lanka [28] and may be an emerging threat to endangered wild populations.

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Early diagnosis of TB in elephants can be difficult. The majority of elephants infected with TB do not show any clinical signs until the disease is advanced when weight loss, weakness, anorexia, and decreased physical activity may be observed [19, 22]. The intradermal tuberculin test used to diagnose TB in domestic livestock is not accurate in elephants [19, 22]. Isolation of the TB organism from culture of samples collected using a trunk wash technique is considered the gold standard but has several limitations, such as difficulty in handling the trunk to obtain a proper sample, poor sample quality due to contamination, and low case detection rate (largely due to intermittent shedding) [21].

Recently developed serological assays are an alternative to screen elephants for TB. The ElephantTB STAT-PAK[®] Assay (ChemBio Diagnostic Systems, Inc., Medford, NY, USA) which utilizes a cocktail of selected *M. tuberculosis* antigens and a conventional lateral-flow method, was licensed by the United States Department of Agriculture in 2007 [15, 16]. The DPP VetTB[®] Assay licensed in 2012, replaced the ElephantTB STAT-PAK[®]. The DPP allows the test sample and antigen-detecting agent to be delivered independently and includes two test lines containing CFP/ESAT-6 and MPB83 antigens. The two tests show comparable results [8]. There is inter-species as well as intra-species variation in the degree and type of antigen recognition by TB-infected animals, but elephants have been shown to have a robust response to serological response primarily to CFP10/ESAT-6 [17].

Studies in human patients with active TB have shown that the red blood cell (RBC) count, hemoglobin (Hb), platelet count, and mean platelet volume are reduced [10, 29, 38]. A combined platelet count and albumin (plateletcrit-albumin) scoring model associated with increased platelet count and decreased albumin had high sensitivity to detect active TB [14]. The importance of the routine blood hematology and biochemical parameters for assessing health status is recognized, however, they have not typically been used as biomarkers for TB because of the complex physiology of TB [18, 29, 31].

Although reference values for hematology and biochemistry parameters in clinically healthy elephants are published [4, 7, 11, 33], information comparing these parameters between TB-infected and TB-suspect elephants is limited. In a study from a North American facility, the albumin:globulin ratio, mean cell hemoglobin concentration (MCHC), and glucose were found to be significantly lower in TB culture-positive elephants (n=4) whereas other hematology and biochemistry parameters were not significantly different [9]. In a previous study in 2006 from Nepal, no significant association was found between hematology and biochemistry parameters and TB culture results [6]. However, this previous study considered 10 of a total 108 tested elephants to be culture positive based on acid fast positive results, and only four of 10 culture positive elephants were reported to be infected with *M. tuberculosis* based on the niacin test result, thus showing limitation in accurate identification of TB positive elephants.

There are approximately 250 captive elephants in Nepal that are used for wildlife management, conservation research, and eco-tourism. Captive elephants frequently interact with wild elephants and share grazing areas with other wildlife, providing the opportunity for transmission of TB from infected elephants to other hosts, including humans. Assessing the health and monitoring the TB status of captive elephants is critical to managing TB at the elephant-wildlife-domestic livestock-human interfaces. This study aimed to determine the seroprevalence of TB in the captive elephant population of Nepal, compare hematology and biochemistry parameters between seropositive and seronegative elephants, and determine if these parameters are of diagnostic value.

MATERIALS AND METHODS

Study area and population

This study was conducted under the Nepal Elephant Healthcare and TB Surveillance Program, a multi-stakeholder program initiated in 2007. During the study period from February 2008 to June 2009, 153 government and private owned captive elephants from the Chitwan National Park (CNP), Bardia National Park (BNP), Shuklaphanta National Park (SNP), and Parsa National Park (PNP) were screened for TB. Twenty male and 133 female elephants were evaluated. The mean reported age for 138 elephants was 38.5 years (range 2–71 years). The ages of 15 elephants were unknown.

Blood collection

Blood was collected from a caudal auricular vein using a 19-gauge winged IV infusion sets (Jor-Vet, Jorgensen Labs, Loveland, CO, USA) and adapter (Becton-Dickinson and Co., Franklin Lakes, NJ, USA) into EDTA and serum separator tubes (Kendall, Covidien, Mansfield, MA, USA). Hematology and biochemistry analyses were performed within 9 hr of blood collection in most cases; in three cases (elephants from SNP and BNP) serum was frozen at -20°C and EDTA blood was stored at 4°C for 5 days until analysis.

ElephantTB Stat-Pak[®] Assay

The ElephantTB Stat-Pak[®] Assay was performed according to the manufacturer's instructions. Briefly, one full drop (30 μl) of serum and 3 drops (about 100 μl) of the diluent were added to the sample (S) well. The result was read after 20 min. The results were interpreted as seropositive or seronegative according to the presence or absence of a blue solid line in the test (T) area followed by a positive line in the control (C) area.

Hematological and biochemistry examination

Biochemistry analysis was performed using serum from 22 TB seropositive elephants (female=19, male=3, average age 43 years, and age range 14–70 years) from CNP (n=18), BNP (n=2), SNP (n=1), and PNP (n=1). Hematology analysis was performed on 13 seropositive elephants (female=11, male=2, average age=44.3 years, and age range 14–70 years) from CNP (n=9), BNP (n=2), SNP (n=1), and PNP (n=1). As a comparison, biochemistry and hematology analyses were performed on nine seronegative elephants

(female=7, male=2, average age 36.3 years, and age range 6–51 years) from a captive facility within CNP in which the entire herd (n=9) was seronegative. This elephant herd was located in a remote part of the park and had infrequent interaction with other elephant herds. Blood analysis was conducted in the pathology laboratory of B.P Koirala Memorial Cancer Hospital in Chitwan, Nepal. Hematology parameters (hemoglobin, packed cell volume, platelet, white blood cells, and erythrocyte sedimentation rate), except for the erythrocyte sedimentation rate (ESR) were analyzed using a Pentra XL 80 auto analyzer (Horiba). The biochemistry profiles [(Blood urea nitrogen (BUN), creatinine, bilirubin total, glutamic oxaloacetic transaminase/aspartate aminotransferase (GOT/AST), glutamic pyruvic transaminase/alanine aminotransferase (GPT/ALT), Gamma glutamyl transferase (GT), lactate dehydrogenase, total protein, albumin, and globulin) were analyzed using a Dimension RxL autoanalyzer (Dade Behring). The ESR was performed using the wintrobe tube method with 22 seropositive and 63 seronegative elephants in the field laboratory at the Biodiversity Conservation Center of the National Trust for Nature Conservation.

Data analysis

The data was analyzed using Microsoft excel program. The association of TB seroprevalence and hematology and biochemistry parameters was analyzed using the *t*-test.

RESULTS

Seroprevalence of elephants for tuberculosis

Of 153 elephants tested during the study period, 33 (21.56%) elephants were seropositive for TB using the Elephant TB Stat-Pak Assay. The majority of TB seropositive elephants were from CNP (n=29) (Table 1). Thirty of 153 female elephants and 3 of 20 male elephants were seropositive (Table 2). The mean reported age for 31 seropositive elephants was 41.25 years (range 14–59 years). The ages of two seropositive elephants were unknown. The mean reported age for 124 seronegative elephants was 37.63 years (range 2–71 years). The ages of 13 seronegative elephants were unknown. Elephant age was not found to be associated with TB seroreactivity in this study ($P>0.05$).

Hematology and biochemistry analysis

The tested hematology parameters (hemoglobin, packed cell volume, platelet count, white blood cell count, and erythrocyte sedimentation rate) were not significantly different ($P>0.5$) between TB seropositive and seronegative elephants (Table 3). The biochemistry parameters of bilirubin (0.31 ± 0.04 and 0.27 ± 0.02), total protein (8.2 ± 0.57 and 8.72 ± 0.61), and lactate dehydrogenase (245.59 ± 117.65 and 368.11 ± 64.23) were significantly different ($P<0.05$) between TB seropositive and seronegative elephants, whereas BUN, creatinine, GOT/AST, GPT/ALT, gamma GT, and albumin were not significantly different. The difference of globulin (6.75 ± 0.57 and 7.42 ± 0.66) between the two groups of elephants was highly significant ($P<0.01$), and the mean value of albumin was higher in the TB seronegative group, indicating that total protein value may be an early indicator for TB (Table 4). The difference between the mean ESR values of the seropositive (48.3 ± 11 mm/hr) and seronegative (49.7 ± 10.5 mm/hr) groups was not statistically significant ($P>0.05$).

DISCUSSION

In this study, we report the TB serology results of 153 captive elephants in Nepal tested from February 2008 to June 2009. We found that 33 (21.56%) elephants were seropositive using the ElephantTB Stat-Pak[®] Assay. Similar seropositive rates have been reported in captive elephants in Malaysia 15.87% (10/63) [24], Thailand 30% (18/60) [35], and Laos 36% (29/80) [12].

Although the majority of seropositive elephants were from CNP (n=29), there were cases in other national parks. This suggests TB may be widespread among the captive elephant population of Nepal. We found a higher seroprevalence of 22.3% (29/130) among elephants from CNP compared to a 2006 study in which 13% (15/115) of CNP elephants were seropositive, and 12 seropositive elephants were common in both studies [20]. The majority of seropositive elephants were female (n=30, 90%) when compared to males (n=3, 10%), but gender was not directly associated with TB seroprevalence in this study. Although the age of elephants was not significantly associated with serological status, the average age of seropositive elephants (41.25 years) was slightly higher than seronegative elephants (32.7 years) ($P=0.25$) suggesting older elephants may be susceptible to TB in Nepal.

In another study from Nepal [26], six deceased elephants had TB antibody responses months to years before culture-confirmed TB diagnosis, indicating the usefulness of serosurveillance for TB in elephants.

Hematology values [hemoglobin, Packed cell volume (PCV), platelet, total white blood cells (WBC) count, and erythrocyte sedimentation rate (ESR)] were not significantly different between TB seropositive and seronegative elephants ($P>0.05$) and were similar to published reference values [4, 7, 11, 33]. A recent study has reported that TB-infected elderly elephants have lower hemoglobin than the reference values related to the physiology of iron in chronic TB infections [30]. We found similar hemoglobin values in both our groups which were also similar in age and may not have been in the chronic stage of TB infection. The ESR is considered a non-specific indicator of inflammation and infection and can be an indicator of pulmonary TB infection in humans [1], usually associated with chronic infection [34]. We did not find an association between the ESR and seroreactivity in our study. Our results show that bilirubin, total protein, and lactate dehydrogenase (LDH) values were significantly different ($P<0.05$); and globulin value was highly significantly different ($P<0.01$) between the two groups of elephants. The lower total protein and globulin values in TB seropositive elephants are likely due to infection with TB although nutritional status and parasite

Table 1. Seroprevalence of tuberculosis in captive elephants in four national parks of Nepal

Location	ElephantTB Stat-Pak [®] Assay			Total
	SeroNegative	SeroPositive	Invalid test	
	Number (%)			
Chitwan National Park	96	29	5	130 (84.9)
Bardia National Park	10	2		12 (7.84)
Parsa National Park	4	1		5 (3.26)
Shuklaphanta National Park	5	1		6 (3.92)
Total	115 (75.16)	33 (21.56)	5 (3.2)	153 (100)

TB, tuberculosis.

Table 2. Association of sex with seroprevalence of tuberculosis in captive elephants of Nepal

Sex	ElephantTB Stat-Pak [®] Assay				P-value
	Negative	Positive	Invalid test	Total	
	Number (%)				
Male	16	3	1	20 (13.07)	0.74
Female	99	30	4	133 (86.92)	
Total	115 (75.16)	33 (21.56)	5 (3.26)	153 (100)	

TB, tuberculosis.

Table 3. Comparison of hematology value of tuberculosis seropositive and seronegative elephants

Parameters	Seropositive			Seronegative			P-value
	N	Range	Mean ± SD	N	Range	Mean ± SD	
Hemoglobin (Hb) g/dl	13	12.5–15.1	13.26 ± 0.68	9	9.7–14.0	12.66 ± 1.33	0.18
PCV (%)	13	34–43	36.76 ± 2.51	9	27–39	35.01 ± 3.45	0.22
Platelet count (/mm ³)	13	183,000–668,000	387,636.4 ± 136,476.6	9	282,000–550,000	419,000 ± 77,857	0.27
WBC (/mm ³)	13	8,000–75,000	19,292.31 ± 17,247.53	9	11,900–18,800	14,944.44 ± 2,396.93	0.46
ESR (mm/hr)	22	19–58	49.3 ± 10.5	63	6–61	49.4 ± 10.7	0.91

PCV, packed cell volume; WBC, white blood cells; ESR, erythrocyte sedimentation rate; SD, standard deviation.

Table 4. Comparison of biochemistry values between tuberculosis seropositive and seronegative elephants

Parameters	Seropositive (n=22)		Seronegative (n=9)		P-value
	Range	Mean ± SD	Range	Mean ± SD	
BUN (mg/dl)	2.0–8.0	9.78 ± 3.53	7.0–13.0	10.06 ± 2.17	0.82
Creatinine (mg/dl)	0.6–1.6	1.06 ± 0.22	0.8–1.2	0.96 ± 0.12	0.22
Bilirubin total (mg/dl)	0.23–0.40	0.31 ± 0.04	0.22–0.32	0.27 ± 0.02	0.03*
GOT/AST (U/l)	12.0–40.0	23.45 ± 7.24	18–36	26 ± 6.36	0.36
GPT/ALT (U/l)	11.0–19.0	14.5 ± 1.94	13–16	14.33 ± 1.00	0.81
GammaGT (U/l)	2.0–18.0	8.71 ± 4.54	7.0–24.0	11.33 ± 5.12	0.19
Lactate dehydrogenase (U/l)	70–570	245.59 ± 117.65	254–452	368.11 ± 64.23	0.02*
Total protein (g/dl)	7.0–10.0	8.20 ± 0.57	8.0–10.0	8.72 ± 0.61	0.03*
Albumin (g/dl)	0.9–1.7	1.45 ± 0.22	1.1–1.5	1.30 ± 0.14	0.07
Globulin (g/dl)	6.0–8.0	6.75 ± 0.57	6.4–8.7	7.42 ± 0.66	0.009**

* $P < 0.05$, ** $P < 0.01$. BUN, blood urea nitrogen; GOT/AST, glutamic oxaloacetic transaminase/aspartate aminotransferase; GPT/ALT, glutamic pyruvic transaminase/alanine aminotransferase; GammaGT, Gamma glutamyl transferase; SD, standard deviation; U/l, units/liter.

load may also affect these values. However, albumin was found to be slightly higher in TB seropositive elephants ($P=0.7$). Further monitoring of total protein, globulin, and albumin is necessary to know their association with elephant TB. We found that LDH was significantly higher in TB seronegative elephants, although LDH is reported to be associated with inflammation and is higher in human TB patients [32]. We also found a significant elevation of bilirubin in TB seropositive elephants while ALT, AST, BUN,

and creatinine were not significantly different between the groups, indicating that liver function and kidney function tests may not be directly related with elephant TB in this study. Further monitoring of LDH and bilirubin levels in elephants is necessary to better understand association with TB infection in elephants. Many factors including stages of TB infection, age groups, reproductive status, nutrition, husbandry practices, and other medical conditions may affect these blood profiles. Our findings suggest that low total protein and globulin show a correlation to TB in captive elephants of Nepal. However, we should point out that the range of certain biochemical parameters that were significantly different between seropositive and seronegative elephants did have narrow ranges (for example bilirubin, globulin, and total protein). Thus, the potential of these parameters as a direct biomarker for TB diagnosis is limited in this study. Furthermore, we should cautiously interpret these parameters as these analyses were conducted at one time point between two groups of elephants that did not differ in their apparent clinical health. Further studies from captive elephants of Nepal and other captive elephants from different geographic locations should be conducted to accurately assess these blood parameters to monitor elephant TB.

One of the important limitations of serosurveillance of elephant TB is its inability to determine the clinical stage of TB disease in elephants. The ElephantTB Stat-Pak[®] Assay cannot distinguish between active and latent TB infection. Most of the seropositive elephants in this study were in apparent good health and their body condition scores were similar to seronegative elephants, but the state of TB disease was unknown. We recommend assessing these blood parameters in TB culture-positive elephants to further elucidate their diagnostic value. We recommend further studies to accurately assess association of LDH and bilirubin with TB.

In conclusion, we report that 33 (21.56%) of captive elephants from four national parks were seropositive for TB. The TB prevalence rate is increasing in the captive population in Nepal suggesting ongoing TB transmission. In conjunction with serological testing, blood parameters may be useful in determining TB status in elephants and should be included in elephant TB surveillance programs.

CONFLICTS OF INTEREST. The authors have nothing to disclose.

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