

## Seroprevalence of Tuberculosis in Captive Asian Elephants in Nepal

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### ABSTRACT

*Tuberculosis (TB) is an infectious zoonotic disease, characterized by the development of tubercles resulting in caseation and calcification in the lungs. In elephants, causative agents *Mycobacterium tuberculosis* and *Mycobacterium bovis* result in chronic weight loss, anorexia, and weakness with occasional dyspnea or coughing. TB has been a major threat to elephants in Nepal. To date, 17 elephants have died in Nepal due to TB alone. Therefore, this study was undertaken to screen the TB seropositive elephants in Nepal. A cross-sectional study was conducted to determine the seroprevalence of tuberculosis (TB) in captive Asian elephants in four protected areas of Nepal from 22 November 2015 to 17 April 2016. The serum samples were examined for tubercle bacillus antibodies by DPP Vet TB Assay. Out of 92 elephants, 10.87% of elephants were found reactive to the bacterium *Mycobacterium tuberculosis* complex. There was a non-significant difference in seroprevalence of TB in captive Asian elephants for the sex, age, and location ( $P>0.05$ ). In a follow-up study, we found that 4 TB seropositive elephants from our study died due to TB and displayed granulomatous tuberculosis lesions with the caseous mass in the lungs on post-mortem examination. Government and non-government stakeholders should jointly formulate effective plans and policies to eradicate tuberculosis from elephants in Nepal.*

**Keywords:** *Captive Asian elephants, DPP Vet TB assay, Tuberculosis (TB), Zoonotic disease*

### INTRODUCTION

Tuberculosis (TB) is a chronic disease that affects humans and different animal species throughout the world. Elephant TB has been described in domesticated elephants as long as 2000 years ago in Vedic literature (Iyer, 1937). *M. tuberculosis* is the predominant disease-causing agent of TB in elephants while few infections with *M. bovis* and non-tuberculous *Mycobacterium* species are also recorded (Paudel et al., 2014). Because the disease is chronic and insidious, antemortem signs are rarely present and most TB diagnoses are made upon postmortem examination.

Nepal has more than 200 captive elephants that are in proximity to humans and domestic animals (Pradhan et al., 2011). These captive elephants have been used in patrolling, and tourism; thus, making them more prone to the possible transmission of *M. tuberculosis* from humans as Nepal has a higher load of TB (WHO, 2014). The first case of elephant TB in Nepal was reported in 2002. Since then, 17 elephants have died due to TB alone. During 2006-2007, elephant TB was

the major threats in the elephants in Nepal, when many elephants were diagnosed positive with TB. Routine surveillance and timely treatment were able to curve the number of TB-positive elephants in Nepal. It has been reported that 61 positive TB cases have been successfully treated to date. However, there has been a recent surge in the mortality of elephants due to TB. TB infected captive elephants is not only of concern for a zoonotic disease but also for the sound health of other wildlife species such as wild elephants, rhinoceros, etc. TB has also been reported in the one-horned rhinoceros in Nepal (Thapa et. al., 2015). Therefore, there is an impending need for the proper screening of TB in captive elephants in Nepal.

In this study, the serological method, Chembio DPP Vet<sup>®</sup> TB Assay (Chembio Diagnostic Systems, Inc., Medford, NY), that detects antibodies to ESAT-6/CFP 10 and MPB83 antigens was used for screening the TB in the captive Asian elephants. DPP VetTB<sup>®</sup> assays are licensed by the United States Department of Agriculture and have been used for TB screening in several Asian elephant range countries (Abraham et al., 2008; Ong et al., 2013). Besides that, serological methods are relatively simple, accurate, inexpensive, non-invasive, and non-dependent on the detection of mycobacteria. Thus, the objective of this study was to determine the seroprevalence of Nepalese captive elephants from the different protected areas of Nepal by using DPP Vet<sup>®</sup> TB Assay.

## **MATERIAL AND METHODS**

### ***Study site***

A cross-sectional study was carried out in the four protected areas, viz- Chitwan National Park (CNP), Parsa National Park (PNP), Bardia National Park (BNP), and ShuklaPhata National Park (ShNP) of Nepal from November 2015- April 2016. The geographical location of the study area is shown in Figure 1.

### ***Study animal***

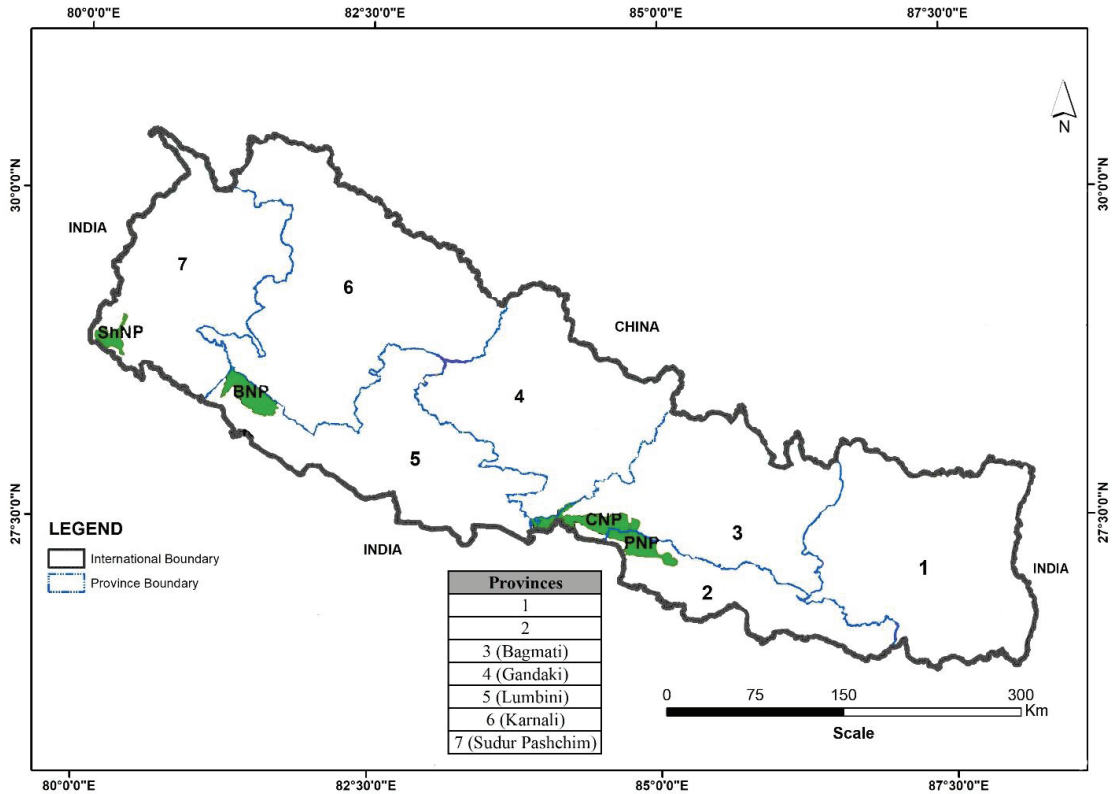
A total of 92 captive Asian elephants, mainly used for patrolling and tourism purposes were used in this study. The location wise number of elephants were 57, 11, 17, and 7 from CNP, PNP, BNP, and ShNP respectively. The age of the study elephant ranged from 2 to 77 years of age.

### ***Sample collection and serology testing***

About 5 ml of blood was collected from the caudal auricular vein of the elephant by using the 19-gauge winged IV infusion set. Thus, collected blood was centrifuged at 3000 rpm for 30 min; serum was separated and aliquoted into cryogenic vials. TB testing was carried out on the same day while the remaining serum was stored at -20 °C for future analysis.

The TB testing was done on the serum sample by using the DPP VetTB<sup>®</sup> assay (Chembio Diagnostic Systems, Inc., Medford, NY). The DPP Vet<sup>®</sup> TB Assay is a single-use immunochromatographic rapid test for detection of antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in serum. The TB testing by using the DPP VetTB<sup>®</sup> assay was carried out by following the manufacturer's instructions. Briefly, 5 µl of serum was loaded in the center of the round sample + buffer well followed by adding buffer in the same well. 5 minutes after loading the sample in well 1, around 4 drops of the buffer were added to the square buffer well 2. Finally,

the test result was read 15 minutes after the addition of the buffer into the buffer well 2. Results were interpreted by observing the test lines in control and test areas



**Figure 1.** Protected conserved areas that were considered in this study.

### Statistical analysis

TB seroprevalence was estimated by calculating the percentage of seropositive out of the total number of tests in the study. The data were analyzed by using MS-EXCEL 2010 and Open Epi version 3. Chi-square test and Fisher Exact Test were used to test the association between variables and results obtained. The P-value of less than 0.05 was considered statistically different.

## RESULTS

Out of 92 captive Asian elephants tested during the study period, 10 elephants (10.87%) were seropositive for TB. Thus, the overall seroprevalence of TB was found 10.87% in the captive Asian elephant. The seroprevalence of the TB in the male captive Asian elephants and that of the female captive Asian elephants were 9.38% (3/32) and 11.67% (7/60) respectively. There was no significant difference in the prevalence of TB between male and female captive Asian elephants ( $P > 0.05$ ) (Table 1).

Among different locations, the seroprevalence of the TB in the captive Asian elephant was found 12.28% (7/57), 5.88% (1/17), 9.09% (1/11), and 14.29% (1/7) in the CNP, BNP, PNP, and ShNP

respectively. No such difference was observed in the seroprevalence of TB among different locations ( $P>0.05$ ). There was no significant difference in the seroprevalence of the TB in the captive Asian elephants among different age groups ( $P>0.05$ ). The seroprevalence of the TB was 3.56%, 20%, 13.33% and 25% for (0-20), (20-40), (40-60) and (60-80) years respectively in the captive Asian elephants.

**Table 1.** Prevalence of TB among sex, age, and location in captive elephants

	No of elephants	TB negative	TB positive	Seroprevalence %	P-value
<b>SEX</b>					0.7366
Male	32	29	3	9.38	
Female	60	53	7	11.67	
<b>LOCATION</b>					0.8793
CNP	57	50	7	12.28	
BNP	17	16	1	5.88	
PNP	11	10	1	9.09	
ShNP	7	6	1	14.29	
<b>AGE (years)</b>					0.8221
<20	39	38	1	2.56	
20-40	15	12	3	20	
40-60	30	26	4	13.33	
60-80	8	6	2	25	
<b>TOTAL</b>	92	82	10	10.87	

We also did a follow-up study on the TB positive elephants from our study and found that 4 (2 male and 2 female) elephants that were tested positive in our study have already died, while remaining positive (6) are kept inside the national park and are closely monitored. To date, they display normal sound health and are only used for normal work. The dead elephants were emaciated and weak and showed granulomatous tuberculosis lesions with the caseous mass in the lungs on post-mortem examination (Figure 2).



**Figure 2.** Granulomatous tuberculosis lesion with the caseous mass in the lungs of dead elephants.

## **DISCUSSION**

The overall seroprevalence of TB in captive Asian elephants in this study was observed 10.87%, which was lower than study in the Peninsular Malaysia (20.4%)(Ong et al., 2013) and in India (15%) (Abraham et al., 2008). The lower seroprevalence in this study may be the result of continuous monitoring and surveillance for TB in captive elephants over a period by the Nepal Government, National Park, other NGOs/INGOs. Nepal TB Project was launched in 2006. This project conducted regular screening and treatment for TB in all captive Asian elephants of Nepal. Moreover, Nepal Elephant TB and Management Action Plan (2011-2015) has been instrumental in decreasing the TB infection in Nepalese captive elephants by thorough surveillance and treatment. In fact, Nepal Elephant TB Control and Management Action Plan is the first such program in the Asian elephant range country (Mikota et al., 2015).

In terms of a different location, the seroprevalence of TB was found higher in the captive elephants from the CNP region (12.28%). This finding was in agreement with the previous study done by (Mikota et al., 2015), where they found 15 TB reactive cases from a total of 115 tested captive elephants (13%). CNP, a world heritage site, harbors the highest number of captive elephants in Nepal. Captive elephants in the CNP are used for patrolling, rhino census, and for tourism. Besides,



these captive elephants often intermingle with domestic cattle during grazing in the buffer area. Thus, these elephants are at high risk of getting TB from mahouts, tourists, and domestic animals. This might be one of the reasons for the higher prevalence of TB in captive elephants from CNC. The breeding facility is located adjacent to CNP, where captive elephant cows are bred with wild elephant bulls. So, constant surveillance of TB among the captive elephants is warranted not only to minimize the TB cases in the captive elephants but also to prevent the spread of TB in wild elephants. Although, the prevalence of TB was higher in female captive elephants as compared to males with no significant difference between them. This study also revealed a higher prevalence of TB in the older captive elephants (more than 20 years) than the younger captive elephants (less than 20 years).

In this study, DPP Vet<sup>®</sup> TB Assay was used for determining the seroprevalence of TB in the captive elephants. DPP Vet<sup>®</sup> TB Assay has been used to screen TB in several species of captive cervids and is approved by the USDA Bovine TB Eradication Program (Lyashchenko et al., 2018). Paudel et al. (2018) performed the 3 different serological tests - the Elephant TB STAT-PAK<sup>®</sup>, DPP VetTB<sup>®</sup> Assay, and MAPIA along with the culture test on the eight suspected or confirmed TB elephant samples. They reported DPP Vet<sup>®</sup> TB Assay was reactive in all eight cases which were also positive for the culture test. This shows the effectiveness of the DPP VetTB<sup>®</sup> Assay that we used in our study.

Serological tests detect antibodies present in serum using antigen-antibody response. It does not access the clinical state of TB. Therefore, other rigorous diagnostic methods such as culture and molecular identification by using PCR should be carried out along with the serological methods. Considering the recent increase in the mortality of elephants due to TB, routine screening, and monitoring of the elephants are required.

## CONCLUSION

The overall seroprevalence of TB in captive Asian elephants in Nepal was found to be 10.87%. In a follow-up study, we also found that 4 positive elephants in our study died due to TB. The remaining positive elephants should be constantly monitored, and further molecular diagnosis approaches need to be considered. Moreover, government and non-government stakeholders should jointly formulate effective plans and policies for the eradication of tuberculosis from elephants in Nepal.

## ACKNOWLEDGEMENTS

This research was supported by the USAID Hariyo Ban Program by providing a DPP Vet<sup>®</sup> TB Assay kit. I thank Mr. Chitra Bahadur Khadka and Mr. Kiran Rijal, veterinary officer and veterinary technician respectively from CNP who provided support for sample collection.

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