Foreword


With the support from Elephant Care International and the collaboration of the National Trust for Nature Conservation (NTNC) and World Wildlife Fund (WWF) Nepal, Nepal has instituted the first elephant tuberculosis surveillance and management program in Asia. This program enhances the health of our elephants, supports our goal to conserve endangered species in our parks, and mitigates the threat of tuberculosis to them.

At this stage, I would like to thank all the funding agencies and conservation partners including the United States Fish and Wildlife Service Asian Elephant Conservation Fund, Elephant Care International, NTNC, WWF Nepal, the Abraham Foundation, the American Veterinary Medical Foundation, the Columbus Zoo, the Oklahoma City Zoo, the Humane Society of the United States, the Mazuri Fund, and the Walter J. Ernst Memorial Fund for extending financial and technical support to institute the tuberculosis prevention program in Nepal. Elephant Care International deserves special thanks for its assistance in fund raising, setting up the tuberculosis program in Nepal, capacity building, and sharing Nepal's success stories with global elephant conservation agencies.

The plan will be implemented through the Department of National Parks and Wildlife Conservation in close collaboration with its conservation partners. I look forward to sharing our success with other elephant range countries of Asia. I would like to appreciate the efforts made for this cause. Thank you.

Yuba Raj Bhusal
Secretary
Ministry of Forests and Soil Conservation
The Department of National Parks and Wildlife Conservation is proud in bringing out the Nepal Elephant Tuberculosis Control and Management Action Plan amidst an alarming health condition of captive elephant due to tuberculosis. This action plan is expected to be a practical guideline to minimize the increasing risk from tuberculosis to not only the captive elephants but equally to wild population of elephants and other wildlife species, tourists, elephant handlers, and staff working in the close proximity of elephants. A number of national and international partner organizations including the National Trust for Nature Conservation (NTNC), Elephant Care International (ECI - USA), the Institute of Agricultural and Animal Sciences (IAAS), World Wildlife Fund Nepal (WWF) and the Zoological Society of London (ZSL) have directly or indirectly extended their support to produce this Plan.

Preparation of this Plan would not have been possible without the untiring efforts from individuals representing different institutions. First of all, I would like to thank Dr. Susan Mikota (ECI), Dr. Shant Raj Jnawali and Dr. Ghana Shyam Gurung for their untiring efforts and involvement from the draft phase to the final version of this Plan. Shiv Raj Bhatta, Dr. Narendra M. B. Pradhan, Gopal P. Upadhyaya, Megh B. Panday, Phanindra Kharel, Naresh Subedi, provided their valuable input during various stages of plan preparation. Dr. Kamal Gairhe, Dr. Jeewan Thapa and Dr. Sarad Paudel provided technical inputs. Barbara Vincent from ECI assisted during the Plan preparation workshop.

Special thanks also go to Dr. I. P. Dhakal (IAAS), Christy William (WWF), Hank Hammatt (ECI), Dr. Gretchen Kaufman (Tufts University, USA), Dr. Michele Miller (USA), Dr. Basu Pandey, Dr. Richard Kock (ZSL) and Dr. Joel Maslow (USA) for their valuable input on the draft document.

Finally, I would like to request all the individuals, conservation partners, community based organizations, funding agencies to join us in our mission to create an elephant tuberculosis free Nepal and secure the long term survival of our country’s unique biodiversity.

Sincerely

Krishna Prasad Acharya
Director General
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Summary

The Nepal Elephant Tuberculosis (TB) Control and Management Action Plan (NETCMAP) is based on a goal of minimizing all factors leading to the risk of TB transmission from captive elephants to the wild. The program will be launched by the Ministry of Forests and Soil Conservation - Department of National Parks and Wildlife Conservation (DNPWC) with technical support from national and international conservation partners including the National Trust for Nature Conservation (NTNC) Nepal, the Buffer Zone Management Committees (BZMCs) Nepal, Elephant Care International (ECI) USA, World Wildlife Fund (WWF) Nepal, and the Hotel Association Nepal (HAN). The plan will be implemented under close supervision of a veterinarian with adequate knowledge of animal TB.

Issues identified in captive elephant TB control and management include: i) Risk of tuberculosis to the captive elephants, ii) Risk of transmission of TB from captive to wild population of elephants, rhino and other wildlife species, iii) Risk of transmission of TB from human to elephant and from elephant to human, iv) Possibility of impact of TB on wildlife tourism in Nepal, v) Insufficient technical and managerial capacity, vi) Insufficient financial resources, and vii) Inadequate research on elephant and free ranging wildlife species. A range of activities have been proposed to address these issues.

All captive elephants in Nepal will be screened for TB. Based on diagnostic test results all elephants will be placed in a management group as described in Section 1.2 TB Testing Procedures and Management Groups.

It is recommended that elephants entering Nepal from India must first be screened for TB using the Elephant TB Stat-Pak® test. Only elephants that are non-reactive on the screening test should enter Nepal. Elephants that do not have proper documentation (written and photograph) from a licensed veterinarian of pre-entry testing using the Elephant TB Stat-Pak® test will be tested once they are in Nepal. Any elephants that are positive on the Elephant TB Stat-Pak® test will be required to leave or undergo treatment as described in this protocol.

Elephants will be treated for TB according to protocols described in Section 1.5. All elephant handlers and other staff working in close proximity to elephants will be screened annually for TB using free services available in Nepal. In the case of new elephant handlers, thorough screening for TB will be undertaken before they begin work with elephants. Annual certificates will be issued to facilities in compliance with this program (Appendix II Plan Certificates).
All elephants in Nepal will be microchipped. Microchipping will take place at the time of TB testing for those elephants that are not already microchipped. All privately-owned elephants will be licensed and registered. Licenses will be issued by DNPWC. License renewals will be issued in conjunction with regular TB testing and documentation of compliance with the TB Plan specified in this document. Records of licensure, registration, and microchips will be maintained at TB program office at Sauraha. Duplicate records will be maintained at DNPWC headquarters.

A full necropsy will be performed on all elephants that die in Nepal. Postmortem examination will include a comprehensive gross necropsy, histopathology, and culture for TB. Postmortem examination can provide a definitive diagnosis and important information for the TB program. Refer to the Nepal Elephant Postmortem Protocol in Appendix III. Completed necropsy reports must be sent to the TB Program Office at NTNC within 30 days.
Abbreviations

AREAS  Asian Rhino and Elephant Action Strategy
BCC   Biodiversity Conservation Centre
BZ    Buffer Zone
BZMC  Buffer Zone Management Committee
CNP   Chitwan National Park
CVL   Central Veterinary Laboratory
DLSO  District Livestock Service Office
DoLS  Department of Livestock Service
DOTS  Directly Observed Therapy Short-Course
DNPWC Department of National Parks and Wildlife Conservation
DPP   Dual Pathology Platform
ECI   Elephant Care International
HAN   Hotel Association Nepal
IAAS  Institute of Agriculture and Animal Sciences
LAMP  Loop-mediated Isothermal Amplification
MAPIA Multi-antigen Print Immunoassay
MOU   Memorandum of Understanding
NATA  National Anti-Tuberculosis Association
NP    National Park
NTNC  National Trust for Nature Conservation
NVSL  National Veterinary Services Laboratories
PA    Protected Area
PCR   Polymerase Chain Reaction
TB    Tuberculosis
USDA  United States Department of Agriculture
USFWS United States Fish and Wildlife Service
WR    Wildlife Reserve
ZSL   Zoological Society of London
Definitions

**Atypical mycobacteria:** see non-tuberculous mycobacteria

**Culture positive for M. tb complex:** Isolation and identification of *M. tuberculosis* complex organisms from any body site using standard mycobacterial methods.

**Culture positive (M. tb complex) elephant:** An elephant from which a *M. tuberculosis* complex organism has been isolated. A culture positive elephant is considered positive until it has met the treatment requirements as outlined in this document.

**Dual Path Platform (DPP) Vet’TB test™:** A field test used to confirm Elephant TB Stat-Pak® test results. The DPP Vet’TB test™ detects antibodies to specific TB antigens. Elephants that are reactive on the Elephant TB Stat-Pak® and react on line 2 or lines 1 and 2 of the DPP Vet’TB test™ are considered TB-infected based on current scientific data (Greenwald *et al.* 2009). Infected elephants may or may not be shedding TB at the time of testing. The test was developed by Chembio Diagnostic Systems Inc (Medford, NY, USA).

**Elephant TB Stat-Pak ® test:** A qualitative screening test for TB in elephants which detects antibodies to *M. tuberculosis* and *M. bovis* in elephant sera, plasma or whole blood. During development, the Stat-Pak was referred to as the “RT” or “Rapid Test.” The Elephant TB Stat-Pak ® test is a USDA-approved product. (See Lyashchenko *et al.* 2005, Lyashchenko *et al.* 2006, Greenwald *et al.* 2009).

**Exposed:** An elephant that 1) has had direct contact (touching or shared air space) with a known TB-infected elephant or human or 2) that has been kept in a stall adjacent to an infected elephant or which 3) has had any other close association with an infected elephant or human.

**Genotyping assay:** A technique for the identification and analysis of polymorphism in certain types of repeat units in DNA. Restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR) are examples of genotyping techniques.

**Incidence:** The rate at which a certain event occurs, for example, the number of new cases of a specific disease occurring during a certain period.

**Herd:** A group of elephants maintained on common ground. Alternatively, two or more groups of animals under common ownership or supervision that are geographically separated, but that may have an interchange or movement of animals or personnel (such as elephants owned by DNPWC).

**Loop-mediated isothermal amplification (LAMP):** A nucleic acid amplification assay that has been used to detect TB in human patients in Nepal (Pandey *et al.* 2008).
**Multi antigen print immunoassay (MAPIA):** A lab-based confirmatory test for the Elephant TB Stat-Pak, currently available only in the USA (Lyashchenko et al. 2000, Lyashchenko et al. 2006, Greenwald et al. 2009).

**Mycobacterium:** A genus of bacteria in the family Mycobacteriaceae.

**Mycobacterium avium (M. avium):** A non-tuberculous mycobacteria that causes TB in birds and occasionally humans and other species. *M. avium* may be isolated from non-clinically affected elephants and is usually considered as an environmental contaminant.

**Mycobacterium bovis (M. bovis):** The primary causative agent of TB in cattle, bison, and deer; may also infect other mammals including pigs, humans, primates, elephants, and non-captive ungulates.

**Mycobacterium tuberculosis (M. tuberculosis):** The primary causative agent of tuberculosis in humans; may also affect a variety of animals, including primates, pigs, cattle, dogs, parrots, elephants, and rhinos.

**Mycobacterium tuberculosis complex (M. tb complex):** A group of mycobacteria which includes *M. tuberculosis, M. bovis, M. africanum, M. microti, M. canetti, M. caprae,* and *M. pinnipedii.* A vaccine strain derived from *M. bovis* (*M. bovis* BCG) is sometimes included in this group.

**N-95 mask:** A mask that is certified to protect against TB when properly fitted.

**Necropsy:** A postmortem (after death) examination to determine the cause of death.

**No isolation:** Absence of growth of *M. tuberculosis* complex organisms from trunk wash, feces, tissue, or other samples using standard mycobacterial culture methods. Failure to isolate organisms may be due to the following reasons:
- the animal is not infected
- the animal may be infected but TB organisms were not present in the sample submitted for analysis
- sampling error (culture overgrowth by contaminating organisms, inadequate sample, laboratory error)
- improperly handled or shipped sample

**Non-reactive:** A negative finding on a test.

**Non-tuberculous mycobacteria (NTM):** Mycobacteria that do not generally cause granulomas. Most NTM are saprophytes found in soil or water. They are typically non-pathogenic but may occasionally cause disease in humans and animals including elephants. It is also referred to as “atypical” mycobacteria or “Mycobacteria other Than TB” (MOTT).

**Nucleic acid amplification test:** A technique that amplifies entities such as DNA or RNA.

**PCR (polymerase-chain reaction):** A nucleic acid amplification technique in which specific sequences of
nucleic acid (DNA or RNA) are replicated, allowing for detection of target sequences.

**Prevalence:** The total number of cases of a specific disease in a given population at a given time.

**Quarantine:** Enforced segregation to prevent the spread of an infectious disease.

**Reactive:** Presence of response; in the context of serological testing for TB in elephants, a reactive result indicates that an antigen-antibody reaction has occurred.

**Segregation:** Separation from others. In context of this protocol, segregation at the home facilities means to keep an elephant apart from other elephants by a minimum distance of 15 meters and to maintain an empty stall on either side of the segregated elephant. Segregation can also be at a location remote from the home facility such as the segregation facility in Kasara.

**Sensitivity:** A measure of the ability of a test to identify infected animal (i.e. the percentage of true positive results). Sensitivity is the frequency of a positive or abnormal test result (e.g. a test that is outside of the reference interval) when a disease is present (i.e. the percentage of true positive results). Sensitivity = \( \frac{TP}{(TP + FN)} \times 100 \) where TP = true positive; FN = false-negative.

**Shedding:** Active excretion of live TB organisms, usually in respiratory secretions. Indicates that an elephant is infectious to other animals or humans. Shedding is determined by culture, PCR, or other technologies that detect TB organisms.

**Specificity:** A measure of the ability of a test to identify non-infected animals (i.e. the percentage of true-negative test results). Specificity is the frequency of a negative or “normal” test result when a disease is absent (i.e. the percentage of true-negative (TN) test results. Specificity = \( \frac{TN}{(TN + FP)} \times 100 \).

**Triple sample method:** A method of sample collection whereby 3 samples are obtained on separate days.

**Trunk wash and modified trunk wash:** Procedures used in elephants to obtain a sputum sample for culture or PCR.

**TB-infected:** An elephant that is reactive on the Elephant TB Stat-Pak® test and the DPP Vet’TB test™ or from which TB complex organisms have been identified by culture or molecular techniques.

**TB suspect:** An elephant that is reactive on the Elephant TB Stat-Pak® test.

**Tuberculin skin test:** A screening test for TB used in humans and some animals. The test cannot accurately diagnose TB in elephants and it is not recommended.
Part I

TB Management
1.1 Introduction

Background

Elephants belong to the Family Elephantidae in animal kingdom and are the largest mammalians among terrestrial animals. Elephants are believed to have evolved some 50 million years ago. Out of about 350 species under 44 Genera of this family, only two genera (Elephas and Loxodonta) and three species remain today – the Asian elephant (*Elephas maximus*), the African bush elephant (*Loxodonta africana*), and the African forest elephant (*Loxodonta cyclotis*). The Asian elephants are distributed in 13 Asian countries: Nepal, India, Bhutan, Bangladesh, China, Myanmar, Thailand, Indonesia, Laos, Vietnam, Cambodia, Malaysia, and Sri Lanka.

Taming of Asian elephant began in the Indus valley (now in Pakistan) around 4,000 years ago. Taming of elephants was done for various purposes. Elephants were trained and used in warfare in India, China and Persia over the centuries. In the past, elephants were extensively used across their range in timber industries, particularly in logging and lifting large logs, and safari hunting. Today, elephants in south and south-east Asia are used in tourism and protected area management. In some places, elephants are still used in circuses because of their ability to be trained to perform, and also because of their impressive size. Elephants in Hindu communities are regarded as a symbol of Lord Ganesh.

In Nepal, elephants were used for Royal hunting safaris for Royalty. Elephants were also kept privately and were used as a symbol of status and means of transportation. After the creation of the Protected Areas, elephants have been extensively used in patrolling, surveillance, research and monitoring, means of transportation and wildlife tourism. Wild elephants in Nepal are confined in the protected areas mainly in Suklaphanta Wildlife Reserve, Bardia National Park, Chitwan National Park, Parsa Wildlife Reserve, and nearby forest areas. However, elephants from the West Bengal State of India frequently visit the forest areas in Jhapa, Morang, Udayapur and Sunsari Districts in the eastern lowland. A low number of private elephants are also reported from Kapilbastu, Rautahat and Sunsari Districts.

Elephants are prone to various infectious diseases including tuberculosis, anthrax, haemorrhagic septicaemia, foot-and-mouth disease, rabies, tetanus, encephalomyocarditis virus, pox, salmonellosis, elephant endotheiotropic herpes virus infection, and others. Among these, tuberculosis (TB) is a chronic, debilitating disease that affects captive elephants world-wide. TB is a threat to captive elephants, wild elephants, other wild and captive animals, and humans.

TB was first identified in captive elephants in Nepal in 2002 (Gairhe 2002). Between 2002 and 2009, there were seven deaths in which TB was diagnosed among government and NTNC-owned elephants. Surveillance for TB in elephants in
Nepal began in 2006. Culture and experimental serological tests were used to screen elephants at this time. One of these tests - the ElephantTB Stat-Pak® (Chembio Diagnostics, Inc, Medford, New York, U.S.A. www.chembio.com) was subsequently licensed by the United States Department of Agriculture (USDA) in 2007 as a screening test for TB in elephants.

Cultures collected in 2006 and evaluated at the National Tuberculosis Institute (NTI) in Nepal and the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, U.S. proved non-diagnostic due to contamination, storage, and transport issues. The same problems were encountered when elephant respiratory samples were submitted to the Nepal Anti-Tuberculosis Association (NATA) in subsequent years. These and other issues, such as intermittent shedding, limit the value of culture as a primary diagnostic technique. In Sweden, only 7 of 189 respiratory samples collected from 5 elephants confirmed TB-infected at postmortem were culture-positive (Moller et al. 2005). In Thailand, TB was isolated from only 2 of 60 respiratory samples collected from confirmed TB-infected elephants (Angkawanish et al. 2010).

Culture as well as molecular diagnostic techniques such as PCR is nonetheless valuable and improving the methods to apply these techniques to elephants remains an important goal. Collaborations have been established with the Nepal Anti-tuberculosis Association (NATA) and the Center for Molecular Diagnostics – Nepal (CMDN) for this purpose. In 2009-2010, these laboratories confirmed Mycobacterium tuberculosis, the human strain of TB, as the causative agent in three elephants in Nepal.

TB can be transmitted from humans to elephants and from elephants to humans. In 2008, over 100 elephant handlers were tested for TB, but no cases of TB were detected. TB is a long-term disease and previous handlers, no longer employed may have been the source of infection for the three M.tb-infected elephants. These elephants may also have acquired TB from other infected elephants. It is presumed that elephants in Nepal may also harbor the bovine strain of TB however laboratory capacity to diagnose M. bovis is limited and this has not yet been confirmed. This is an area for further research.

Tuberculosis can be caused by a number of mycobacterial species and many of these bacteria infect multiple hosts. The recognition of TB in elephants is stimulating a shift in thinking about how disease is perceived. Generally zoonoses are the concern of the public health community but TB is an anthroponosis (an infectious disease in which a disease causing agent carried by humans is transferred to other animals) highlighting the need for involvement of both animal and human health professionals and agencies. A further complication in the human community has been a rise in HIV/AIDS; a disease often associated with tuberculosis which it promotes through immune suppression of the human host and by which Nepal is increasingly suffering. Therefore, a more holistic view of tuberculosis surveillance and control is needed. This requires a much broader, “One-Health” (Human - Livestock - Wildlife) approach which is gaining momentum in the global health community. This paradigm takes into account the entire epidemiology of the disease, examines all the susceptible species, carriers and likely drivers of infection and epidemics. Part of this elephant TB strategy must be to integrate with the other health professions concerned with livestock and humans, bringing the perspective and knowledge gained from the elephant and wildlife experiences. A One Health Nepal initiative is in the conceptual stage and TB is considered a perfect example to provide proof of concept and better management, diagnosis, control and prevention of the disease amongst all animal species and most importantly, human beings.
Treatment for TB amongst elephants began in 2008. Exposure to known cases of TB and a reactive Elephant TB Stat-Pak® result were initially used as the basis for treatment. The current decision tree includes the DPP “VetTB™” test (see 1.5. TB Treatment Protocol).

Routine surveillance of elephants and their handlers together with early diagnosis and treatment are essential to control TB. It is hoped that these guidelines will serve as a science-based model for other Asian elephant range countries.

The Nepal Elephant Tuberculosis (TB) Control and Management Action Plan (NETCMAP) is based on a goal of minimizing the risk of TB transmission from captive elephants to the wild by managing TB at the captive-wild interface. This plan outlines methods to diagnose, treat, and manage TB in elephants in Nepal. The TB control and management program will be launched by the Ministry of Forests and Soil Conservation - Department of National Parks and Wildlife Conservation (DNPWC) with technical support from national and international conservation partners including the National Trust for Nature Conservation (NTNC) Nepal, the Buffer Zone Management Committees (BZMCs) Nepal, Elephant Care International (ECI) USA, World Wildlife Fund (WWF) Nepal, and the Hotel Association of Nepal (HAN). The plan will be implemented under the close supervision of a veterinarian with adequate knowledge on animal TB.

1.2 TB Testing Procedures and Management Groups

The TB Plan Veterinary Officer and Veterinary Technician will be responsible for testing all elephants in Nepal.

Procedure

1. Perform the Elephant TB Stat-Pak® test

2. If Elephant TB Stat-Pak® test is non-reactive the elephant is considered TB-free at the time of testing.

3. If Elephant TB Stat-Pak® test is reactive perform the DPP’ VetTB™ test.

4. If DPP’ VetTB™ test is non-reactive the elephant is TB-suspect.

5. If DPP’ VetTB™ test is reactive the elephant is considered TB-infected.

Management groups

Group 1. TB-free. An elephant that is non-reactive on the Elephant TB Stat-Pak® test. Continue every other year testing with the Elephant TB Stat-Pak®. No segregation or work restrictions.

Group 2. TB-suspect. An elephant that is reactive on the Elephant TB Stat-Pak® test and non-reactive on the DPP’ VetTB™ test. There are two options.

Option 1 (recommended especially for elephants that have contact with wild elephants or rhinos): Initiate prophylactic treatment (described in section 1.5). Elephants in this sub-group should be segregated while they are adjusting to treatment and should not work until they have completed 30 consecutive days of treatment (30 full doses). Elephants that have had a known exposure to a TB infected elephant or human or whose exposure status is unknown should follow Option 1. Following completion of treatment, elephants will be designated as Group 2 - Treated.

Option 2: Test with the DPP’ VetTB™ test at 6 months following the first reactive Elephant TB Stat-Pak® result. If the DPP is reactive at this time, the elephant changes to Group 3. If the DPP is non-reactive at 6 month test again at one year. If the test is non-reactive at one year, monitor annually by the DPP’ VetTB™ test. Elephants
Distribution of captive elephants in Nepal

Legend
- Districts of Nepal
- Protected Areas
- Captive elephants (Confirmed)
- Captive elephants (Potential)

Note:
NP = National Park
WR = Wildlife Reserve
HR = Hunting Reserve
CA = Conservation Area
that have had no known exposure to an infected elephant or human may elect Option 2. Option 2 may also be suitable for old elephants or as a temporary option for pregnant elephants. Elephants in this sub-group should not work and must remain segregated from serologically non-reactive elephants and from wild elephants and other wild ungulates until they have had two non-reactive DPP tests.

Group 2 TB-suspect elephant may or may not be shedding TB organisms. Staff caring for elephants in this group should consider personal protection (see section 1.6).

The combination of a reactive Elephant TB Stat-Pak® test and a non-reactive DPP® VetTB™ test may be due to the one of the following:

1. Early sero-conversion. The elephant is truly infected but seroconversion on the Elephant TB Stat-Pak® has preceded seroconversion on the DPP. TB is a chronic disease and the body’s antibody response may vary with the stage of the disease which can affect test results. Further research to better understand the elephant immune system is on-going (Landolfi et al. 2009, Landolfi et al. 2010). In almost all cases to date culture positive (shedding) elephants have been reactive on both the Elephant TB Stat-Pak® and the DPP® VetTB™ tests. While elephants that are Elephant TB Stat-Pak® reactive and DPP® VetTB™ non-reactive are probably less likely to be shedding they may convert to DPP® VetTB™ reactive status and / or become TB culture-positive and shed TB organisms at any time. This has occurred in a several cases including a case in Nepal.

2. Prior treatment for TB.

3. In rare instances, the Elephant TB Stat-Pak® may produce false positive results. The Elephant TB Stat-Pak® may cross-react with certain NTM such as M. szulgai, which caused the death of two African elephants (Lacasse et al. 2007). It has been demonstrated however, that the Elephant TB Stat-Pak® and DPP® VetTB™ tests do not react with 11 other NTMs nor with M. avium (Greenwald et al. 2009). In one study, only 4 of 147 cases in the U.S. and Europe were considered to be false-positives (Greenwald et al. 2009).

**Group 3. TB-infected.** An elephant that is reactive on the Elephant TB Stat-Pak® and the DPP® VetTB™ tests and /or from which M. tuberculosis complex organisms have been identified by culture or molecular techniques.

- Segregate according to procedures in Section 1.4.
- Collect respiratory samples for culture and molecular diagnostics (see procedure below) before initiating treatment.
- If culture is positive perform drug sensitivity testing.
- Initiate treatment as soon as possible and suspend work and public contact until the elephant has completed 90 consecutive days of treatment (90 full doses). Treat according to protocols in section 1.5
- Elephants that have a positive culture are known to be shedding, are a higher risk to transmit TB and should be segregated for the duration of treatment.
- Institute precautions to protect human health.
- Document that treatment is carried out in accordance with these guidelines. The TB Plan Veterinary Officer will be responsible for monitoring treatment.
- Monitor post-treatment with culture and molecular techniques as the Elephant TB Stat-Pak® test will remain reactive for some time.
See section 1.5 for details.

- Elephants that have completed treatment will be designated as **Group 3 - Treated**.

**Note:** TB-suspect and TB-infected elephants should be treated under most situations. The decision to treat old or pregnant animals should be made on a case by case basis. While very old elephants with long-standing infections are unlikely to be cured, treatment may prevent shedding and older elephants (>50 years) in Nepal have undergone treatment with minimal to no side effects.

TB drugs have also been administered to lactating elephants with no side effects to mother or calf. In one case a pregnant elephant under treatment aborted but it is unknown whether this was associated with the TB drugs or other causes. TB drugs are routinely administered to pregnant humans with no ill effects.

If a decision is made to withhold treatment of a TB-infected elephant, the elephant should be maintained in permanent segregation for the remainder of its life and contact with wildlife strictly prevented. Because of their highly social nature, visual and auditory proximity to other elephants is acceptable as long as the distance precludes any direct contact with respiratory secretions, the elephant is cared for by a separate handler (wearing personal protection) and separate cleaning utensils are used for the infected elephant. All untreated TB-infected elephants are a risk to other elephants, humans, and wildlife.

**Group 4. Untested:** An elephant that has not yet been tested for TB. Untested elephants already in Nepal (such as calves) should be tested as soon as possible. Elephants entering Nepal should be tested prior to entering Nepal by a licensed veterinarian experienced in performing the Elephant TB Stat-Pak® test or by the TB Plan Veterinary Officer.

Written documentation must include the elephant’s name; age; sex; owner’s name and contact information; veterinarian’s name and contact information; and test result. A photograph of the elephant and a photograph of the test labeled with the elephant’s name and date of the test must be included. The TB Plan Veterinary Officer has the authority to test new elephants or to re-test elephants to verify test results upon arrival in Nepal.
Elephant TB Stat-Pak Test

Stat-Pak Non-reactive

TB Free [Group 1]

Test every 2 years; no segregation or work restrictions

Option 1
Segregate; prophylactic treatment

No work until 30 consecutive doses

Monitor post-treatment with yearly DPP [Group 2 Treated]

Option 2
Repeat DPP at 6 and 12 months

DPP Non-reactive

DPP reactive

DPP reactive [Group 2]

Perform DPP VetTB test

DPP Reactive [Group 3]

Collect respiratory samples

Culture negative

DPP Non-reactive

Monitor yearly with DPP

Move to Group 3

Culture positive

DPP reactive

Treat; Segregate and no work until 90 consecutive doses

Treat; segregate for duration; no work

Monitor post-treatment with culture, PCR, and LAMP every 6 months for 2 years then yearly [Group 3 Treated]
Respiratory Sample Collection Procedures

The Nepal Elephant TB Control and Management Action Plan has established collaborations with the Nepal Anti-tuberculosis Association, the Center for Molecular Dynamics-Nepal (CMDN), and the experts in this field to improve methods to identify TB bacterial shedding in elephants. The following procedures may change as new knowledge is gained.

Loop-mediated isothermal amplification (LAMP) is a molecular technique that has been used to diagnose TB in humans in Nepal (Pandey 2008). It is accurate in humans and may prove to be an effective diagnostic tool for elephants but should be performed in conjunction with culture and PCR. Collect samples in the afternoon after the elephant has been active and moving around.

Collection of oropharyngeal swab for culture
1. Wear N95 mask and exam gloves.
2. Place a culturette on the end of the aluminum rod designed for this purpose.
3. Swab the oro-pharyngeal region.
4. Place the swab in the tube and compress the end to release the transport media.
5. Label with the elephant’s name, project ID #, collection date, and facility abbreviation.
6. Refrigerate; ship to CMDN as soon as possible. CMDN will forward to NATA.

Collection of oropharyngeal swab for PCR and LAMP
1. Wear N-95 mask and exam gloves.
2. Place a sterile cotton swab on the end of a plastic extension rod.
3. Swab the oro-pharyngeal region.
4. Place the swab in the pre-labeled, buffer-filled nunc tube.
5. Label with the elephant’s name, project ID #, facility abbreviation, and collection date.
6. Refrigerate; ship to CMDN as soon as possible.
1.3 Description of Diagnostic Methods

a. The Elephant TB Stat-Pak® test is a blood antibody test. A reactive test indicates probable TB infection. Confirmation with other tests is ideal - when possible and as discussed above. A non-reactive test indicates no infection and a TB-free status at the time of testing. The Elephant TB Stat-Pak® test is used as the standard routine TB screening test for elephants in Nepal. The test is administered by the TB Plan veterinarian.

b. The DPP VetTB™ test is a blood antibody test used to confirm the Elephant TB Stat-Pak® test. The Elephant TB Stat-Pak® and DPP VetTB™ tests have been shown to be early predictors of infection and may be reactive months to years in advance of detection by culture (Greenwald et al. 2009). This provides the opportunity to treat elephants before they shed and infect other elephants, humans, or wildlife.

c. Culture is used to detect elephants that are actively shedding TB organisms. Respiratory samples obtained by collecting trunk discharges are submitted to a laboratory that is qualified to test for mycobacteria (this requires specific procedures). A positive culture confirms that an elephant is actively infected. Positive isolates should be tested to confirm if the organisms were *M. tuberculosis* or *M. bovis*. Drug sensitivity testing (DST) for anti-tuberculosis drugs (isoniazid, rifampin, and ethambutol) should be performed.

Note that culture has inherent limitations. Failure to isolate the organism does not rule out infection. False-negative results may occur 1) if the elephant is infected but organisms are not shed on the day of sample collection or 2) contaminated trunk wash samples cause other organisms to overgrow. False-negative results allow TB disease to progress and the infected elephant is a source to spread TB to other elephants, humans, or wildlife. Culture is expensive and many samples may be needed to detect infection. In Sweden, of 189 trunk wash cultures collected from 5 elephants confirmed TB-infected on postmortem, only 7 samples were positive (Moller et al. 2005).

Newer technologies that prove to be more reliable, sensitive, or cost effective for detecting TB organisms in trunk wash or other secretions will be incorporated into this plan as they become available.

d. PCR (polymerase chain reaction) is a type of nucleic acid amplification test (NAAT). NAATs facilitate the identification of pathogens by amplifying and detecting specific nucleic acid sequences. PCR assays to detect TB in elephants in Nepal are under investigation by the Center for Molecular Dynamics-Nepal (see Kay et al. 2011).

e. LAMP (loop-mediated isothermal amplification) assay is a type of NAAT that allows detection of trace amounts of DNA under isothermal conditions. It has been used to diagnose TB in humans in Nepal (Pandey et al. 2008) and is under investigation for use in elephants.

1.4 Segregation Procedures

Segregation is an important management tool. Segregation from wild elephants and other wildlife (e.g. rhino) is considered a priority to prevent TB transmission to endangered wildlife populations. Segregation can also minimize transmission to other captive elephants and to humans. The goal of segregation is to prevent healthy animals from coming into contact with respiratory droplets or other secretions from infected elephants.
Segregation should be instituted as soon as test results indicate. If elephants are to be treated, they should be rested and not used for work or tourism until they have completed 30 consecutive days of treatment (Group 2) or 90 consecutive days of treatment (Group 3). If very old elephants are to be maintained under permanent quarantine they should never be used for work or tourism.

Segregation should ideally take place in a separate location (such as the Kasara segregation facility for government elephants). Private elephants may be segregated at their home facilities if the following guidelines are strictly observed:

a. Segregated elephants should be kept at an appropriate distance (minimum 15 meters) from other animals within the camp and should not be bred.

b. Segregated elephants should be housed such that the adjacent stalls on either side are empty. These elephants should be maintained on a high plane of nutrition. Gram and produce (carrots, pumpkins, sugar cane etc.) should be added to the diet along with the close monitoring of their daily rations.

c. Separate tools should be used to clean and feed segregated elephants and they should be handled last.

d. Elephant handlers should wear N-95 masks when working in close proximity to the elephant and should wash their hands after handling, cleaning, or feeding.

1.5 TB Treatment Protocol

General Considerations

The government will arrange for treating government-owned elephants. Private owners will be responsible for treating privately-owned elephants. Treatment protocols are based on established treatment regimens successfully used in the U.S. Treatment plans for individual elephants will be developed under the supervision of the TB Plan veterinarian. Treatment protocols will be updated as new information becomes available.

The goal is to prescribe TB treatment for elephants in Nepal using the currently available scientific information with the hope of curing the disease. The intent is to strive to achieve the currently recommended dosages with allowance to adjust the dosages for each individual elephant as needed to achieve compliance while minimizing side effects.

It is extremely difficult to understand the severity of TB disease that an individual elephant may have. It may not be possible to completely cure TB in elephants with advanced disease (>50% of the lung involved). So far, no standard measurements have been developed for “complete cure” at this time. See post-treatment monitoring guidelines below.

Drug doses are best determined by obtaining an accurate scale weight. A portable weighing scale is available at the TB Plan office at NTNC. The scale requires a platform for the elephants to stand on. There are currently platforms at NTNC, Kasara, the Chitwan Hattisar, and Bardia.

If scale weighing is not possible, the weight may be estimated using the chest girth method. The chest girth should be measured 3 times and averaged to ensure accuracy. The chest girth is measured immediately behind the front leg. The weight is calculated from the following formula (Hile et al. 1997):

\[
\text{Weight in kg} = 18.0 \times (\text{Heart Girth in cm}) - 3336.
\]

Group 1 elephants have no work restrictions.
Group 2 elephants that elect prophylactic
treatment should be segregated and restricted from work until they have completed 30 consecutive full doses of anti-TB drugs.

Group 3 elephants (Elephant TB Stat-Pak® reactive / DPP® VetTB™ reactive) should be segregated and restricted from work and public contact until the elephant has completed 90 consecutive doses of anti-TB drugs. Culture positive elephants potentially have more advanced disease and should be segregated and restricted from work for the duration of treatment.

Majority of the elephants have tendency of refusing medications. The TB drugs are bitter-tasting and many tablets are required. Prophylactic treatment lasts nine months and full TB treatment lasts one year so it is important to take time to slowly condition the elephant to accept a new routine before starting medication. Once the elephant accepts the new routine they are more likely to complete the entire treatment without a problem. Each elephant is different and requires patience and ingenuity to determine the best method for each individual elephant. The following methods have been used successfully in Nepal. Note that the partial doses suggested below during the conditioning period do not count toward the full dose goal.

Method 1. Direct administration in small molasses balls or bananas

Begin training elephants to accept small balls of molasses with no medication. These should be small enough to be placed towards the back of the elephant’s tongue so that they swallow them without chewing. Each elephant should receive about 100-150 small molasses balls each day in the morning before other food. Once the elephant routinely accepts the un-medicated molasses balls, the drugs may be added gradually as described below. Place only 1-2 pills in each molasses ball. Once the medications are reliably accepted at the full dose it is best to give them all at once in the morning before the elephant eats a lot of food. If this does not work then the drugs can be divided so that the elephant receives 1/2 of the pills in the morning and 1/2 in the evening.

Small pieces of banana (with skin), each containing 1-2 pills can be used in place of molasses and may work better for some elephants. Sugar should not be used as it interferes with the absorption of INH.

Method 2. Direct administration in large molasses balls (or dana/ kuchi)

Some elephants have accepted multiple tablets in large molasses balls or in their regular dana/ kuchi.

Method 3. Direct administration in the mouth

A few elephants have been trained to open their mouths widely and will accept the pills being tossed directly into their mouths. The pills should be followed by a liter of water to make sure that they swallow.

Introduce INH-RIF at 80% of the calculated dose. Place 1-2 pills in small molasses balls (or banana pieces). Try to place in the back of the mouth so that the elephant does not crush and taste the drugs. Place several pills if using large molasses ball or dana/kuchi.

Once INH and RIF have been accepted for 3 days in a row, increase to 90% of the calculated dose.

Once INH and RIF at 90% of the calculated dose are accepted for 3 days in a row, increase to 100% of the calculated dose. If there are any problems noted at 100% of the dose, back down to 90%.

For Group 3 elephants it is important to add EMB as soon as possible after the INH and RIF are
being consumed reliably. Start EMB at 80% of the calculated dose. If there are no problems after 3 days, increase to 90% of the calculated dose, then to 100% of the calculated dose.

**Method 4. Rectal administration.**

Rectal administration is used only for elephants that absolutely cannot be trained to accept oral medications. This method is more costly. For Group 3 elephants another drug (such as ciprofloxacin) must be added or substituted for RIF as RIF is not absorbed well rectally. The procedure is as follows:

Protocol for Rectal administration of anti-tuberculosis drugs in elephants:

a. Place 400 ml of tap water in a jar with a lid.

b. Add the calculated number of tablets.

c. Shake the bottle well until the drugs are completely dissolved. Isoniazid is easily dissolved; ethambutol takes more time.

d. Place a long PD (pregnancy diagnosis) glove on one hand and a regular exam glove over it.

e. Lubricate with soap or other lubricant.

f. After the elephant lies down in either sternal or sleeping position, remove all the accessible fecal boluses from the rectum.

g. Insert one end of a stomach tube into the rectum and slowly pour the drug solution into the funnel at the other end of the tube.

h. Drug administration is facilitated by squeezing the pump located in the middle of the tube.

i. When drug administration is finished clear the tube by flushing it with ~ 100 ml of water.

j. Slowly remove the tube and hand.

k. Maintain the elephant in the treatment position for at least 5 minutes to give the drug time to be absorbed. If the elephant stands up too quickly some of the drug may be expelled.

l. After the elephant stands up, observe to see if any drug has been expelled.

m. Record observations in log book.

n. Only fully retained doses count towards the required number of doses to complete treatment.

**Management of side effects:** Elephants may experience side effects while on TB drugs. Loss of appetite is the most common. If loss of appetite is observed, stop the medications for 1-3 days (or until the appetite improves). When resuming treatment give 25% of the pills the first day, 50% the next day, 75% the 3rd day and the full dose on the 4th day. Be sure and record the change in the number of pills on the elephant’s treatment record. These partial doses do not count toward the full treatment goal.

If elephants display side effects that do not resolve by withdrawing medications for a few days it is advisable to submit blood for a CBC and serum biochemistry tests. In Chitwan the Cancer Hospital will receive elephant samples. The TB drugs may occasionally cause anemia or changes in liver enzymes which can be detected by blood tests. In elephants that have repeated problems with loss of appetite or other side effects, the drugs can be administered at double the dose but given every other day. This is called pulse therapy and has been used successfully in the U.S.

Other signs that may occur (less commonly) include: fever, muscle weakness or limb pain, unsteady gait, skin rash, visual or hearing disturbances. If any of these signs are observed, contact following TB Plan Veterinary Officer.

**Human health:** See Section 1.6
Record keeping: Keeping accurate records is very important. Each elephant under treatment will have a log book to record how much medication is consumed and enter comments about treatment. The log book will be provided by the TB Plan and the TB Plan Veterinary Officer will explain how to use the log. Instruct staff to be honest in reporting whether elephants have consumed all or only a portion of the medication. This information will help the TB Plan Veterinary Officer to assess treatment and make any adjustments if needed. The TB Plan Veterinary Officer may review the log book at any time. Upon completion of treatment the log book will become part of the elephant’s permanent record maintained in the TB Plan office.

Monitoring: The TB Plan Veterinary Officer will monitor all government elephants under treatment. Private veterinarians who are treating elephants will report at least monthly to the TB Plan veterinarian who will stay informed of their treatment progress, offer advice, and maintain a record on the elephant. All privately owned elephants that are treated should also have a log book in which daily notes of the amount of drug offered and the amount consumed are recorded. At the completion of treatment a copy of the log book will become part of the elephant’s record in the TB Plan office to document compliance with the program.

A. Protocol for Management Group 2: TB-suspect elephants (Prophylactic treatment)

Elephants in Group 2 receive two drugs for 9 months.

Oral therapy:

1. Isoniazid (INH) at a dosage of 5 mg/kg for 270 full doses
2. Rifampin (RIF) at a dosage of 10 mg/kg for 270 full doses

Rectal therapy:

1. Isoniazid (INH) at a dosage of 5 mg/kg for 270 full doses
2. Ethambutol (EMB) at a dosage of 30 mg/kg for 270 full doses

Post-treatment monitoring

The Elephant TB Stat-Pak® test may remain reactive even after treatment. Elephants that have been treated prophylactically should be monitored annually using the DPP. If the DPP becomes reactive the elephant may have been re-infected. An elephant with a reactive DPP shifts to Group 3 and should undergo full TB treatment.

B. Protocol for Management Group 3: TB-infected elephants

Elephants in Group 3 receive three drugs for two months followed by two drugs for another 10 months.

1. Isoniazid (INH) at a dosage of 5 mg/kg for 360 full doses
2. Rifampin (RIF) at a dosage of 10 mg/kg for 360 full doses
3. Ethambutol (EMB) at a dosage of 30 mg/kg for 60 full doses given the first 2 months of treatment (60 doses)

Elephants in Group 3 must receive 3 drugs for treatment. Administering fewer drugs has been shown to result in treatment failure. Other drugs may be prescribed based on positive cultures and drug sensitivity testing.

The first 60 doses of all three drugs should be administered within a period of 90 days (i.e. no more than 30 days of refused medication as a combination product. Each tablet contains 300 mg of INH and 600 mg of RIF.
should occur). If more than 30 days of interrupted treatment occur, treatment must start over. Only full doses count toward the total of 60 doses.

Treatment is continued for an additional 10 months with INH-RIF. The total number of doses for the entire treatment is 360 full doses. The entire treatment should be completed within 15 months. This allows for refused medication days and periods of interruption that may be needed if side effects occur.

**Completion of Treatment:** Treatment is complete when an elephant has consumed 60 full doses of EMB and 360 full doses of INH-RIF. There is currently no single test that confirms cure. Weight gain, improved exercise tolerance, improved breathing, and other signs may be used to evaluate treatment response. Other parameters that signify a response to treatment are under investigation.

**Post Treatment Policy:** After successful completion of the prescribed treatment (no greater than 15 months) an elephant will be released back into its home facility and/or resume normal activity. For the first two years post-treatment, respiratory samples for culture, PCR, and LAMP should be submitted every 6 months (triple sample method). After two years, respiratory samples for culture, PCR, and LAMP should be submitted annually (triple sample method).

Note that the Elephant TB Stat-Pak® may remain positive even after treatment, so cannot be used to determine re-infection or treatment failures in elephants that have been treated. The MAPIA declines with treatment (Lyashchenko et al. 2006) and it is expected that the intensity of the reaction on the DPP VetTB™ test may also change post-treatment, however further research in this area is needed. Post-treatment monitoring guidelines may change as new information or tests become available.

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**Section 1.6 Human Health Considerations**

TB can be transmitted between elephants and humans (Michalak et al. 1998). It is recommended that elephant handlers and other exposed staff wear N-95 masks when working in close contact with TB positive elephants. N-95 masks are special masks that are certified to be protective against TB. The mask must be secured tightly around the face to be effective. Wearing masks for the duration of treatment is ideal; at minimum masks should be worn for the first 3 months. Handlers should be instructed to wash their hands after working directly with suspect or infected elephants. All new elephant handlers or other staff working in close contact with elephants should be screened for TB before starting employment. An annual human TB screening program for all staff is essential as exposure from outside sources may occur at any time. Human TB testing services are widely available in Nepal. Documentation of staff TB testing will be required for elephant owners or facilities to receive Certificates of Compliance.
Part II
Action Plan
2.1 Vision

The vision of the Action Plan is to ensure tuberculosis free captive elephants and contribute in conserving biodiversity at all levels in lowland landscape of Nepal.

2.2 Goals

The goals of this plan are to: i) eliminate TB in captive elephants as well as among the elephant handlers and other staff working in close proximity to elephants, ii) prevent transmission of TB to wildlife, and iii) safeguard tourism from potential risk of elephant TB.

2.3 Objectives

The objectives of this plan are to:

i) institute a sustainable program to diagnose and treat TB among captive elephants and their handlers in Nepal,

ii) establish an effective elephant health monitoring system,

iii) establish a TB education program for elephant staff,

iv) educate tourists, hotel owners, local communities and media,

v) integrate the plan into livestock and human TB surveillance and control initiatives and ensure regular reporting to and from these sectors, with collaborative One Health activities supported.

2.4 Issues, Strategies and Program Activities

Issue I: Risk of tuberculosis to the captive elephants

**Strategy:** Institute and enforce a sustainable testing, segregation, and treatment program for all captive elephants and their handlers, and prevent TB-suspect or infected captive elephants from entering Nepal.

**Program Activities**

1. *Program management*

   The Plan will be managed by DNPWC in collaboration with NTNC, WWF Nepal, BZMC and HAN, Chitwan. DNPWC will seek continued technical support from Elephant Care International (ECI). TB Plan Office will be placed at NTNC’s Biodiversity Conservation Center (BCC) at Sauraha, Chitwan.

2. *Testing*

   All captive elephants in Nepal will be TB tested every one to two years in accordance with the procedures in Section 1.2.
3. *Preventing untested elephants from entering Nepal*

Preventing infected elephants from entering Nepal is one of the most effective methods to control TB. The practice of obtaining elephants from outside Nepal is strongly discouraged. All elephants that do enter Nepal must have a certificate of a non-reactive ElephantTB Stat-Pak® test performed within the previous 30 days by a licensed veterinarian. Written documentation must include the elephant’s name; age; sex; owner’s name and contact information; veterinarian’s name and contact information; and test results. A photograph of the test labeled with the elephant’s name and date of the test must be included. The TB Plan Veterinarian will have the authority to retest (if tested previously) elephants to verify test results upon arrival in Nepal.

TB Plan Veterinary Officer will be available to travel with private owners planning to procure new elephants. The private owner will bear the cost of testing and travel expenses for TB Plan Veterinarian. TB Plan Veterinarian will have the authority to test elephants that enter Nepal untested immediately and independently. Elephant TB Stat-Pak® reactive elephants must leave Nepal. All new elephants must be microchipped for identification.

4. *Elephant events*

All elephants participating in events such as elephant races or polo must have a certificate of a current non-reactive Elephant TB Stat-Pak® test or completion of prophylactic treatment (including post-treatment testing as described under Post-Treatment Policy in section 1.5).

5. *Segregation*

All elephants that are reactive on the Elephant TB Stat-Pak® and DPP® VetTB™ tests will be segregated to prevent potential transmission of TB to other elephants, humans, or wildlife according to segregation guidelines given in segregation procedures.

6. *Treatment*

Elephants will be treated for TB as prescribed above in Section 1.5 using the same drugs shown to be effective for humans. Elephants are treated for a longer period of time as a measure of safety as there is still much that is unknown about TB in this species.

7. *Grazing practices*

Efforts will be made to prevent elephants from grazing in close proximity to captive cattle or buffalo to minimize the risk of TB transmission.

8. *Dung disposal*

The current practice of burning elephant dung in close proximity to elephant stables will be discontinued, as smoke is a respiratory irritant and adversely affects the function of the lungs of both the elephants and their handlers. A practical mechanism will be developed and implemented for dung burning at a sufficient distance to prevent smoke from reaching the elephants and their caretakers or alternative methods of dung disposal will be instituted.

9. *One Health working group*

Establish a One Health Nepal working group with local wildlife, medical and veterinary
authorities and clinicians to devise a comprehensive plan to identify pockets of infection and control or eradicate TB from the local environment.

10. Human TB screening

Humans are a potential source of TB for elephants. Handlers and other staff members working in close proximity to elephants will be TB tested annually. New employees will be tested before beginning work. The TB positive staff members will not be allowed to handle or care for the elephants until they have completed four weeks of DOTS treatment. Medication will be started as soon as the person is diagnosed TB positive.

11. Captive elephant postmortem examination

All captive elephants that die will receive a comprehensive postmortem examination conducted by the TB Plan Veterinarian. Nepal elephant postmortem protocol worksheet is given in Appendix III.

12. Monitoring

The feeding, working, and grazing of TB positive elephants will be closely monitored by the owner and the TB Plan veterinary officer.

13. Adoption of animal welfare code

The five freedom codes of animal welfare will be always taken into consideration. The five freedoms are:

i. Freedom from hunger and thirst
ii. Freedom from thermal and physical discomfort
iii. Freedom from injury and disease
iv. Freedom to express most normal patterns of behaviour
v. Freedom from fear and distress

**Issue II: Risk of transmission of TB from captive to wild population of elephants, rhino and other wildlife species**

**Strategy:** Control TB among captive elephants and institute practices to prevent contact between captive TB-suspect or infected elephants and wildlife species.

**Program Activities**

1. **Segregate and treat infected elephants**

All infected elephants will be segregated and treated as per guidelines described under **Issue I**.

2. **Prevent contact**

All possible measures will be applied to prevent the contact between captive TB-suspect or infected elephants and free ranging wildlife species. Use electric fences or other practical methods to prevent contact between captive TB-suspect or infected elephants and free ranging wild elephants, rhinos and other large as well as medium sized ungulates. Captive TB-suspect or infected elephants will be restricted to specified grazing areas. The segregation plan should be approved by the TB Plan veterinary officer.

3. **Educate handlers**

A regular TB control and management education program will be launched among elephant handlers, caretakers and their family members. For this, a practical guide highlighting the causes of TB, symptoms,
Impact of TB on human as well as animal health, importance of preventing TB transmission to the wild and effective measures to manage TB will be developed, presented orally, and printed in the local language to educate persons directly or indirectly associated with the captive elephants.

4. **Postmortem wildlife surveillance**

Thorough postmortem examinations of all ungulates that are found dead in the park, buffer zone community forests and nearby areas will be conducted by experienced veterinarians. For this, tissues will be collected and deposited in 10% buffered formalin even if carcasses are decomposed and will be submitted for histopathology or tissue bank. Any lesions suspicious for TB should be placed in a secure container (no formalin), and submitted fresh (preferable) or frozen for TB culture. Efforts, wherever possible, will be made to collect postmortem blood, separate and bank serum in the TB Plan lab freezer.

Full postmortem reports (or copies) will be filed in the TB Plan Office, respective Park headquarters and DNPWC. A MOU will be signed among DNPWC, NTNC, IAAS, CVL, ECI, WWF, and Tufts to effectively facilitate implementation of a comprehensive TB management program and minimize the risk of transmission from captive elephants to free ranging elephants and other ungulates.

5. **Opportunistic TB testing in wild elephants**

Blood samples from any elephant that is immobilized will be collected and Elephant TB Stat-Pak® test (and DPP if indicated) will be performed. Serum will also be banked for future use.

6. **Opportunistic TB testing in other species**

Opportunistic TB test will also be performed among other wild ungulates or carnivores that are captured and trapped for other purposes. While the Elephant TB Stat-Pak® test is licensed for elephants, it has been used successfully in a number of other species, including rhinos and wild felids

**Issue III: Risk of transmission of TB from human to elephant and from elephant to human**

**Strategy:** Minimize the risk of transmission from elephant to human and human to elephant through an integrated TB management program.

**Program Activities**

1. **Develop an action plan from the One Health working group and implement, to deal with the root cause of TB infection in the environment, animals and people.**

Research will be necessary and surveillance across the environment to establish the main causes and drivers of TB and these needs to be followed up by a control and management plan involving all key authorities in public and animal health.

2. **Enforcing elephant testing, segregation and treatment plan**

As described in Program Activities under Issue I and Issue II.

3. **Human TB screening**

All elephant handlers (Phanits, Pachhuwas and Mahouts), and other staff working in close proximity to elephants will be TB tested prior to employment and retested.
on an annual basis. TB testing by sputum is available free of charge at numerous health clinics in Nepal. Positive sputum tests will be followed by other diagnostic tests and/or DOTS treatment as determined by human health care professionals at the testing facility. If infected individuals are identified, their families will also be tested. Further investigation will be managed by appropriate public health agencies. Elephant handlers undergoing DOTS treatment will not have contact with elephants until they have completed four weeks of treatment. Proof of current elephant handler testing must be presented for a facility to receive a Certificate of Participation (Appendix V).

2. **Educate tourists and tour agencies**

NTNC TB Program Office staff and DNPWC will collaborate to create and distribute a brochure for tourists and tour agencies explaining how the Nepal Elephant Tuberculosis Control and Management Action Plan will safeguard the health of elephants and tourists.

3. **Educate the media**

NTNC TB Plan Office staff and DNPWC will collaborate with local media (both audio and visual) to highlight the ways in which the Nepal Elephant Tuberculosis Control and Management Action Plan safeguards elephant and tourist health. A short documentary may also be prepared to illustrate the Plan.

**Issue V: Insufficient technical and managerial capacity**

**Strategy:** Develop partnerships to strengthen technical and managerial capacities.

**Program Activities**

1. **Strengthen capacity**

Veterinary facilities particularly targeted to TB control will be developed and capacity of park, zoo, Buffer Zone veterinary clinic veterinarians will be enhanced through hands-on-training, workshop and higher degree study programs. Partnership with national and international experts and organizations will also be expanded to...
hold training workshops to encourage veterinary diagnostic and clinical skills for the surveillance of TB and other diseases.

2. **National level collaboration**

Long term collaboration will be established with NATA to improve respiratory sample collection and culture techniques, and with IAAS and CVL to improve pathology diagnosis. Partnerships will also be built with other organizations in Nepal to develop molecular screening techniques for captive elephants and other free ranging wildlife TB.

3. **Internships**

One or two graduates from IAAS may be invited annually for a short internship period (3-6 months) under the NETCMAP to develop a cadre of young veterinarians in wildlife medicine.

**Issue VI: Insufficient financial resources**

**Strategy:** Create a sustainable funding mechanism to support the Nepal TB Control and Management Action Plan.

**Program Activities**

1. **Annual budget**

Annual recurring budget for the Nepal Elephant Tuberculosis Control and Management Action Plan will be prepared.

2. **Partnership**

Long term financial commitment will be sought from national and international conservation partners including NTNC, WWF Nepal, ECI, ZSL, USFWS etc. to ensure financial sustainability of the NETCMAP.

3. **Basket fund**

A basket fund to ensure a regular supply of financial resources will be created at the field level. Potential sources for the basket fund would be concessionaire fee from the lodges inside Chitwan and Bardia NPs, and revenue diverted back to the captive elephant bearing Protected Areas (Koshitappu, Parsa and Suklaphanta WRs; Chitwan, Banke and Bardia NPs).

4. **Fund raising capacity building**

Fund-raising capacity of DNPWC and partner organizations will be developed to sustain long term financial health through a series of grant-writing training workshops.

**Issue VII: Inadequate research on elephant and free ranging wildlife species**

**Strategy:** Develop partnerships with national and international researchers and research organizations to promote elephant and free ranging wildlife TB research.

**Program Activities**

1. **Research collaboration**

Establish collaborations under the One Health Nepal initiative to bring the various health sectors together and establish a proof of concept study into the TB ecology in the environment of the Terai National Parks and buffer zone human and animal communities.

Collaborations will be established to develop tests that can determine the stage of disease; quickly and economically detect shedding; measure blood levels of TB drugs in elephants; confirm cure; and determine
if elephants have genetic susceptibility or resistance to TB. Additional studies will focus on epidemiology, pathology, and improving treatment methods.

A serum bank has been established at NTNC - BCC at Sauraha that is a resource for new studies. Collection of blood and urine will be continued by the TB Plan Veterinary Officer at the time of regular TB testing so that this resource will continue to grow for further research. Elephant Care International will continue to coordinate this effort. A research review committee will be established to review and approve proposals.

2. Affiliations

Affiliations with researchers established by ECI will be continued. Collaboration will also be established with researchers in other countries by sharing information and database generated from the ongoing TB program. ECI will help to coordinate this effort.

3. Molecular screening

Partnerships will be established with national and regional institutions to develop molecular screening techniques for elephant and other free ranging wildlife TB.
### 2.5 Budget

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<td>40,000</td>
<td>50,000</td>
<td>60,000</td>
<td>70,000</td>
<td>80,000</td>
<td>300,000</td>
</tr>
<tr>
<td>ix. Human TB testing for handlers of TB infected elephants</td>
<td>60,000</td>
<td>60,000</td>
<td>60,000</td>
<td>60,000</td>
<td>60,000</td>
<td>300,000</td>
</tr>
<tr>
<td>x. Purchase of post mortem examination supplies</td>
<td>70,000</td>
<td>30,000</td>
<td>30,000</td>
<td>30,000</td>
<td>30,000</td>
<td>190,000</td>
</tr>
<tr>
<td>Xi. Regulation on free movement of elephant (transborder and in country)</td>
<td>300,000</td>
<td>500,000</td>
<td>600,000</td>
<td>300,000</td>
<td>300,000</td>
<td>2,000,000</td>
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<tr>
<td><strong>Issue II: Risk of transmission of TB from captive to wild population of elephants, rhinos and other wildlife species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Education to handlers on TB transmission from captive elephants to other wild animals</td>
<td>250,000</td>
<td>300,000</td>
<td>150,000</td>
<td>150,000</td>
<td>150,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td>ii. Opportunistic TB testing in wild elephants (Purchase of ElephantTB-Stat Pak® and DP-PvetTB™) and other animals</td>
<td>15,000</td>
<td>30,000</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
<td>195,000</td>
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<tr>
<td><strong>Issue III: Risk of transmission of TB from human to elephant and from elephant to human</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Education program handlers’ families and local stakeholders on transmission of TB from human to elephant and from elephant to human</td>
<td>150,000</td>
<td>170,000</td>
<td>180,000</td>
<td>150,000</td>
<td>190,000</td>
<td>840,000</td>
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<tr>
<td>Action plan for one health program</td>
<td>500,000</td>
<td>300,000</td>
<td>300,000</td>
<td>300,000</td>
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### Issues/Activities

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<tr>
<th>Issues/Activities</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Total (NRs)</th>
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<tr>
<td><strong>Issue IV: Possibility of impact of TB on wildlife tourism in Nepal</strong></td>
<td></td>
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<tr>
<td>i. Certificate program</td>
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<td>25,000</td>
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<td>25,000</td>
<td>250,000</td>
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<tr>
<td>ii. Education to tourist and tour agencies</td>
<td>150,000</td>
<td>250,000</td>
<td>200,000</td>
<td>150,000</td>
<td>150,000</td>
<td>900,000</td>
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<td>iii. Education to the media</td>
<td>75,000</td>
<td>70,000</td>
<td>50,000</td>
<td>50,000</td>
<td>50,000</td>
<td>295,000</td>
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<td><strong>Issue V: Insufficient technical and managerial capacity</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>i. Capacity building program for Veterinarian and Managers</td>
<td>1,500,000</td>
<td>1,200,000</td>
<td>700,000</td>
<td>300,000</td>
<td>300,000</td>
<td>4,000,000</td>
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<tr>
<td>ii. Laboratory expenses at national level laboratories</td>
<td>1,500,000</td>
<td>750,000</td>
<td>750,000</td>
<td>750,000</td>
<td>750,000</td>
<td>4,500,000</td>
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<td><strong>Issue VI: Insufficient financial resources</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>i. Daily office management costs</td>
<td>1,200,000</td>
<td>1,400,000</td>
<td>1,600,000</td>
<td>1,800,000</td>
<td>2,000,000</td>
<td>8,000,000</td>
</tr>
<tr>
<td>ii. Computer, camera, printer, stationary, fax etc</td>
<td>350,000</td>
<td>150,000</td>
<td>150,000</td>
<td>150,000</td>
<td>150,000</td>
<td>950,000</td>
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<tr>
<td>iii. Vehicle purchase and management</td>
<td>4,500,000</td>
<td>450,000</td>
<td>450,000</td>
<td>450,000</td>
<td>450,000</td>
<td>6,300,000</td>
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<tr>
<td><strong>Issue VII: Inadequate research on elephant and free ranging wildlife species</strong></td>
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<tr>
<td>i. Expenditure on the collaborative research</td>
<td>1,000,000</td>
<td>1,000,000</td>
<td>1,000,000</td>
<td>1,000,000</td>
<td>1,000,000</td>
<td>5,000,000</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td>16,625,000</td>
<td>9,935,000</td>
<td>10,455,000</td>
<td>9,285,000</td>
<td>10,610,000</td>
<td>56,910,000</td>
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</table>
Bibliography

In addition to the literatures cited, this list also contains selected TB references. For complete TB bibliography, please visit: www.elephantcare.org


Appendices
Appendix I

Sample Drug Dose and Cost Calculations (Based on May 2010 Prices)

TB drug cost / tablet:
Isoniazid (INH); 300 mg = Rs 2
Isoniazid-Rifampin combination (INH-RIF); 300 mg INH + 600 mg RIF = Rs 8.6
Ethambutol (EMB); 800 mg = Rs 6.1

The following sample calculations are for a 3000 kg cow:

A. Prophylactic Treatment for Group 2 Elephants: two drugs for nine months

1. Oral protocol

INH-RIF:
Dose /day: 5mg/kg/day x 3000 kg = 15,000 mg
Tablets/day: 15,000 mg / 300 mg / tablet = 50 tablets
Total # of tablets: 50 tablets / day X 270 days = 13,500 tablets
Total cost: 13,500 tablets @ Rs 8.6 /tablet = Rs 116,100 (plus replacements for wasted drugs).

2. Rectal protocol (more expensive; use only if oral therapy cannot be managed)

INH:
Dose /day: 5mg/kg/day x 3000 kg = 15,000 mg
Tablets/day: 15,000 mg / 300 mg / tablet = 50 tablets
Total # of tablets: 50 tablets / day X 270 days = 13,500 tablets
Total cost: 13,500 tablets @ Rs 2 /tablet = Rs 27,000 (plus replacements for wasted drugs).

EMB:
Dose /day: 30mg/kg/day x 3000 kg = 90,000 mg
Tablets/day: 90,000 mg / 800 mg / tablet = 112 tablets
Total # of tablets: 112 tablets / day X 270 days = 30,240 tablets
Total cost: 30,240 tablets @ Rs 6.1 /tablet = Rs 184,464 (plus replacements for wasted drugs).

Total cost: Rs 211,464 (plus replacements for wasted drugs).
B. Full Treatment for Group 3 Elephants: three drugs for two months followed by two drugs for 10 months

1. Oral protocol

INH-RIF:
Dose /day: 5mg/kg/day x 3000 kg = 15,000 mg
Tablets/day: 15,000 mg / 300 mg / tablet = 50 tablets
Total # of tablets: 50 tablets / day X 360 days = 18,000 tablets
Total cost: 18,000 tablets @ Rs 8.6 /tablet = Rs 154,800 (plus replacements for wasted drugs).

EMB:
Dose /day: 30mg/kg/day x 3000 kg = 90,000 mg
Tablets/day: 90,000 mg / 800 mg / tablet = 112 tablets
Total # of tablets: 112 tablets / day X 60 days = 6720 tablets
Total cost: 6720 tablets @ Rs 6.1 /tablet = Rs 40,992 (plus replacements for wasted drugs).

Total cost: Rs 195,792

2. Rectal protocol (more expensive; use only if oral therapy cannot be managed)

INH:
Dose /day: 5mg/kg/day x 3000 kg = 15,000 mg
Tablets/day: 15,000 mg / 300 mg / tablet = 50 tablets
Total # of tablets: 50 tablets / day X 360 days = 18,000 tablets
Total cost: 18,000 tablets @ Rs 2 /tablet = Rs 36,000 (plus replacements for wasted drugs).

EMB:
Dose /day: 30mg/kg/day x 3000 kg = 90,000 mg
Tablets/day: 90,000 mg / 800 mg / tablet = 112 tablets
Total # of tablets: 112 tablets / day X 360 days 40,320 tablets
Total cost: 40,320 tablets @ Rs 6.1 /tablet = Rs 245,952 (plus replacements for wasted drugs).

Ciprofloxacin: 5-10mg/kg (empirical dose) or Amikacin (injectable): to be discussed with TB Plan Veterinary Officer on a case-by-case basis.
Appendix II

Plan Certificates

For compliance with a regular testing program:

Certificate of Participation

This is to certify that Name of Hotel or Owner has tested the elephants and mahouts for tuberculosis in compliance with the guidelines of the Nepal Elephant Tuberculosis Management Program administered by Department of National Parks and Wildlife Conservation.

Date: ...........................................
Signature

For elephants that have completed treatment:

Certificate of Completion of Elephant TB Treatment

This is to certify that Name of Elephant (program ID....) owned by Name of Hotel or Owner has completed treatment for tuberculosis in compliance with the guidelines of the Nepal Elephant Tuberculosis Management Program administered by Department of National Parks and Wildlife Conservation.

Date: ...........................................
Signature
Appendix III

Nepal Elephant Postmortem Protocol

Special considerations for TB-infected elephants or TB suspects

A thorough search for lesions of tuberculosis (TB) is encouraged in all elephant necropsies especially elephants that are known to be reactive on the Elephant TB Stat-Pak® and DPP Vet®TB™ tests. Elephant TB is likely to be caused by *M. tuberculosis* or *M. bovis* which are contagious to humans. Wear proper protective apparel, and contain any suspicious organs or lesions as soon as possible. All involved personnel should wear N-95 masks.

**Approach:** During examination of an elephant with unknown, suspicious, or positive TB test history, dissection of the thoracic cavity should always be performed last, and should be done by two people with N-95 face masks and other protective clothing. All other personnel should be dismissed from the area before the thoracic cavity is entered. After the abdominal viscera have been removed, the diaphragm can be cut from its costo-sternal attachments and the lungs palpated from a caudal approach for tuberculosis nodules, as the lobes are being separated from the closely adhered visceral and parietal pleura. The heart, lungs, and associated structures may then be removed “en bloc”.

**Necropsy procedures:** Carefully examine the tonsillar regions and sub-mandibular lymph nodes for TB lesions. These lymph nodes may be more easily visualized following removal of the tongue and laryngeal structures during the dissection. All lymph nodes should be carefully evaluated for lesions since other sites may also be infected (ex. reproductive or gastrointestinal tract). Take any nodes that appear caseous or granulomatous for culture (freeze or place in sodium borate) and histopathology (in buffered 10% formalin).

Search thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Section the nodes and collect in formalin even if no lesions are present. If lesions are present, place half of the lymph node in a 50 ml screw-top tube and submit for culture.

Carefully, palpate the lobes of both lungs from the apices to the caudal borders to detect any firm B-B shot to nodular size lesions. Take numerous (5 or more) sections of any suspicious lesions. Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly. Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.
Nepal Elephant Postmortem Worksheet

Elephant name: _________________________________  TB Program ID # __________

Owner / location: ________________________________

Age: ________  Sex: __________

Estimated or actual weight (Kg): ______

Country of origin: ___________  Captive Born ___ Wild Caught ___ Unknown ___

Phanit: _______________________  Pachhuwa: _______________  Mahout: _____________

Date / time / location of death: ______________________________________________________

Date / time/ location of postmortem: __________________________________________________

Post mortem interval / condition: _____________________________________________________

History:

Gross examination (Describe abnormal; indicate N= normal or NE = not examined):

1. External (physical and nutritional condition, skin, body orifices, temporal gland)
2. Musculoskeletal (muscles, bones, joints)
3. Body cavities (fat stores, pleura, fluid, thymus, lymph nodes)
4. Spleen and Liver
5. Respiratory system (trunk passages, pharynx, larynx, trachea, bronchi, lungs, lymph nodes; (Examine lungs, trachea, tonsil area, and bronchial lymph nodes for evidence of TB; submit any lung lesions for TB culture; culture bronchial lymph nodes for TB even if normal in appearance)
6. Cardiovascular (heart, pericardial sac, vessels, valves, chambers)
7. Digestive (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, pancreas, mesenteric lymph nodes)
8. Urinary (kidneys, ureters, bladder, urethra)
10. Endocrine (thyroids, parathyroids, adrenals, pituitary)
11. Central nervous system (brain, meninges, spinal cord)
12. Sensory organs (eyes, ears)
Check list for fixed tissues: Preserve all tissues in group I and as many tissues in groups II and II as possible. Use 10% buffered formalin at a ratio of ~1 part tissue to 10 parts solution. Cut tissues no thicker than 0.5 cm. Take 2-3 sets. Submit one set for histopathology and bank 2 sets. Freeze 3-5 cm blocks of tissue from lesions and major organs (lung, liver, kidney, spleen, heart) in small plastic bags or tin foil.

Freeze post mortem serum (from heart); urine; and any abnormal fluid accumulations.

Group I

<table>
<thead>
<tr>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>_ heart (sections from auricles, ventricles and valves)</td>
</tr>
<tr>
<td>_ lung (specimens from several lobes)</td>
</tr>
<tr>
<td>_ trachea (may be site for TB)</td>
</tr>
<tr>
<td>_ liver (3 representative specimens and bile duct)</td>
</tr>
<tr>
<td>_ kidney (cortex and medulla of each kidney)</td>
</tr>
<tr>
<td>_ adrenal gland</td>
</tr>
<tr>
<td>_ spleen (representative cross section with capsule)</td>
</tr>
<tr>
<td>_ lymph nodes (submandibular, bronchial, mesenteric, and tonsillar tissue)</td>
</tr>
<tr>
<td>_ pancreas</td>
</tr>
<tr>
<td>_ stomach (several specimens from all areas)</td>
</tr>
<tr>
<td>_ intestines (3cm representative specimen from each region)</td>
</tr>
<tr>
<td>_ thyroid gland (and parathyroid if possible)</td>
</tr>
</tbody>
</table>

Group II

<table>
<thead>
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<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>_ urinary tissue (ureters, urethra, bladder cross section including mucosa)</td>
</tr>
<tr>
<td>_ uterus and ovaries (transverse sections of cervix, uterine horn, and ovaries)</td>
</tr>
<tr>
<td>_ testes and epididymus</td>
</tr>
<tr>
<td>_ prostate, seminal vesicles, bulbo-urethral gland</td>
</tr>
</tbody>
</table>
Group III
__ esophagus
__ tongue
__ salivary gland
__ thymus
__ temporal gland
__ sciatic nerve section
__ muscle
__ bone marrow
__ skin (sections of abdomen, lip and ear pinna)
__ eye (whole eye, incise sclera to allow entry of fixative)
__ mammary gland
__ brain, pituitary gland,
__ spinal cord (sections from cervical, thoracic and lumbar)
__ neonates (umbilical stump and surrounding

Fluids collected: __ serum __ urine __ peritoneal fluid__ other________________

Cultures collected: ____________________________________________

Tissues sent for histopathology to (Pathologist name, address, email): __________________
____________________________________________________________________________

Veterinarian and Assistants: ______________________________________________________

Preliminary Diagnoses: ___________________________________________________________
Appendix IV

Contacts

Central Committee

1. National Trust for Nature Conservation (NTNC)
   Dr. Shant Raj Jnawali (srjnawali@ntnc.org.np)

2. World Wildlife Fund - Nepal
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2. Hotel Association Nepal (HAN)
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3. Institute of Agriculture and Animal Science (Histopathology)
   Dr. D.K. Singh; Phone: 9845023132; Email: dksnl@yahoo.com

4. Chembio Diagnostic Systems, Inc. Medford, NY, USA
   Elephant TB Stat-Pak® and DPP Vet™ TB test™ tests
   Ordering information: Bobbie Coco
   Phone: 1-631-924-1135 x100; Fax: 1-631-924-6033
   Email: BCoco@chembio.com

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   Email: s.dixit@cmdn.org.np
   www.cmdn.org.np