EMERGING DISEASES AFFECTING ASIAN ELEPHANTS

(2023-24)

Photo: Prashant CN

IUCN SSC Species Survival Commission Asian Elephant Specialist Group

Mandate:

Identify and map the (distribution) of potential emerging diseases affecting wild and captive elephants and develop guidelines to treat, minimize, and manage its spread.

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Activities and Findings

Based on collective knowledge, the group agreed that Elephant Endotheliotropic Herpes Virus (EEHV) and tuberculosis (TB) are the two most important infectious diseases of concern for captive and wild elephant in Asia. Anthrax was briefly considered, however there were few reports in the literature concerning anthrax among Asian elephants and outbreaks are also sporadic in nature.

Tuberculosis is an age-old disease of humans and domestic animals but reports of its occurrence among captive Asian elephants are fairly recent. EEHV, on the contrary, is a recently described acute viral haemorrhagic disease but the causative agent must have associated with the host for a fairly long time. An emerging infectious disease is defined as an infectious disease that has newly appeared in a population or that has been known for some time but is rapidly increasing in incidence or geographic range or could increase in the near future. Thought both tuberculosis and EEHV are diseases of increasing concern in Asian elephants, too little is known about them in elephants to outrightly consider them as emerging diseases.

In the absence of formal surveillance systems, there is no way to know the true prevalence of these diseases or the threat that they pose to captive and wild elephant populations. We can only surmise their importance based on published reports and anecdotal information.

Since the mid-1990's, a vast literature about these two diseases have emerged. Every year new reports of their occurrence and spread continue to be published, particularly among captive elephants. Annexure 1 and Annexure 2 list some of the major works on EEHV and TB that have been published as of December 2022.

Elephant Endotheliotropic Herpes Virus (EEHV)

Background

EEHV is a recently recognized virus that is thought to have co-evolved with elephants. There are several subtypes. Asian elephants are susceptible to subtypes EEHV 1 (1a and 1b), EEHV 4, and EEHV 5. Most cases of clinical disease are caused by EEHV 1a which is endemic, and most elephants are considered carriers. No clinical signs are usually associated with primary infection in adults and the virus becomes latent within the elephant's body. However, reactivation with viral shedding can occur and expose immunologically naive individuals.

In such cases, EEHV causes a severe hemorrhagic disease (EEHV-HD). Elephants 1-8 years of age are the most susceptible. Young elephants become infected by direct contact with elephants that are in the reactivation phase and shedding virus in trunk secretions or via mucous membranes. It is thought that elephants < 1 year of age may be protected by maternal antibodies.

EEHV-HD often has an acute onset and can be rapidly fatal even in elephants that are treated early. Clinical signs vary among individuals but may include lethargy, decreased or loss of appetite, cyanosis (blue color), swollen tongue, oral ulcerations, edema of the head, neck, and front legs, small hemorrhages on the tongue or gums, lameness, colic, abnormal sleeping patterns, and other signs.

In cases of EEHV-HD, the virus is thought to attack endothelial cells (cells lining the blood vessels). This leads to the consumption of platelets, capillary leakage, hemorrhages (especially in the heart), shock, disseminated intravascular coagulation, and death.

Diagnosis

Active cases of EEHV in elephants are diagnosed by testing whole blood using conventional (cPCR) or quantitative PCR (qPCR) to detect viral DNA.

Low levels of viremia can sometimes be detected in clinically healthy elephants (Bauer 2018). In these cases, clinical signs, the WBC, and the platelet count can help to determine if and when to treat. qPCR provides a measure of the viral load, while cPCR only determines whether the virus is present or not.

PCR is also used to detect viral shedding in trunk wash secretions in elephants that are thought to shed intermittently throughout their lifetime after both clinical and non-clinical primary infections.

Suspected or confirmed cases that die should undergo a complete postmortem examination. Typical findings include multi-organ hemorrhages and edema, especially in the heart and liver.

Serology can be used to detect antibodies against EEHV which is useful to determine seroprevalence within populations and also identify the possible vulnerable juvenile elephants without antibodies.

There are many EEHV diagnostic labs in Asia (see Annexure 3 for contact details and sample requirements). The names of the officers-in-charge of some of the select 10 these well-known facilities are given in parenthesis:

1. Indian Veterinary Research Institute, Bareilly, UP, India.
2. Advanced Disease Monitoring and Diagnostic Facility (AdMAC), Guwahati, Assam (N.N. Barman)
3. National Trust for Conservation, Biodiversity Conservation Center Molecular Laboratory (Amir Sadaula)
4. Kasetsart University Elephant Herpesvirus Lab (Supaphen Sripiboon)
5. Faculty of Veterinary Medicine, Chiang Mai University, Thailand (Chatchote Thitaram)
6. Veterinary Research and Development Center (Lower Northeastern Region), Surin Province, Thailand (Bopit Puyati)
7. Elephant Hospital, Thai Elephant Conservation Center, Lampang Province, Thailand (Taweepoke Angkawanish)
8. Wildlife Reserves Singapore (Chia-Da Hsu)
9. Syiah Kuala University, Banda Aceh Indonesia (Muhammad Hambal)
10. Satwa Duta Medical Laboratory for Animal Health, Bogor, Indonesia (Adin Priadi)

Guidelines for treatment

Medical management of EEHV-HD cases is complicated and challenging and even captive elephants may require sedation. Therapy must be prompt and aggressive. Facilities housing captive elephants or rescue facilities that may encounter an affected wild elephant should be familiar with the disease, its surveillance, and the treatment. Basic and emergency supplies should be readily available.

There are EEHV groups in North America, Europe, Australia, Africa, and Asia. The EEHV Advisory Group website (eehvinfo.org) offers current information and protocols. Facilities housing elephants are encouraged to visit eehvinfo.org and request membership for easy access to current professional documents.

The Asian EEHV Working Group has developed detailed diagnosis and treatment guidelines for treating elephants suffering from EEHV in Asia (Annexure 4).

Distribution – captive and wild

The first case of EEHV from Asia was reported in a captive Asian elephant in Cambodia in 2006 (Reid *et al.* 2006). As of October 2020, at least 19 publications concerning EEHV in Asia have appeared (See Annexure 1). To date, EEHV has been reported from captive elephants in Thailand,

India, Laos, Nepal, Cambodia, Malaysia and Myanmar, and in wild elephants in Thailand, India, Sri Lanka, and Laos.

Mitigation and management (minimizing spread)

Because EEHV is endemic, and many if not most elephants are carriers, control within captive populations is aimed at close surveillance of elephants in susceptible age groups for early diagnosis and treatment. Daily observations of appetite, behavior, temperature, and urine and feces as well as training for routine examinations and blood draws is recommended. Positive reinforcement training to permit blood draws and weekly monitoring of WBC and platelet counts can facilitate early diagnosis. Early EEHV viremia is often associated with low WBC, monocytopenia, and thrombocytopenia. Healthy baselines should be established for each elephant. Treatment should be initiated when monocytes and platelets suddenly drop. In remote areas where diagnostic facilities are unavailable, aggressive treatment should commence as soon as the mahouts report signs of EEHV. The keepers should be sensitized beforehand and guidelines and drugs on the management and treatment of the disease should be kept ready in every camp. Serology with the determination of antibody levels can serve as an indicator of the susceptibility of a naïve calf to develop EEHV-HD when going through primary infection (Hoornweg et al. 2022).

Necropsy of every unnatural death in captivity is of paramount importance. Many captive elephants are located in remote areas without access to quality veterinary care. Deaths in such camps often go unrecorded, and thus prevalence and distribution of EEHV also go unrecorded. Most owners of captive elephants are not keen on conducting necropsies, and this should be monitored by the authorities issuing the ownership certificates.

Implications for the wild populations:

Like other known emerging infectious diseases (canine distemper in lion and tiger, mycotic Chytrid fungus in amphibians, cutaneous nodules in Irrawaddy dolphins), the impact of EEHV in wild elephant populations is unknown. Published information on the occurrence of EEHV in wild elephants is now available (Zachariah et al, 2013; Barman et al, 2017). Treatment of elephants in the wild is currently impossible, but research projects for non-invasive detection and surveillance of wild elephant populations are underway.

Frequent monitoring for carriers is the key, but active and invasive collection of samples from wild elephant populations for the purpose of monitoring alone is impossible. Meanwhile, the possibility of screening viral DNA in feces is being investigated. All wild caught individuals, particularly orphan calves should be screened for EEHV virus using available tests. Calves rescued from the wild could be held in isolation for a few days till the results of screening are available. A reliable serological test, when made available, will help to determine the presence of maternal antibodies in displaced calves upon admission.

Unlike tuberculosis which is communicable to many mammalian species in the wild, EEHV is a disease endemic to the elephants, and thus its impact will be limited to elephants alone. However,

elephants being a gregarious species, individuals will always be at the risk of contracting the infection from conspecifics that are carriers.

Tuberculosis

Background

TB is bacterial disease. In elephants, it is mainly caused by *Mycobacterium tuberculosis* (*M. tb*), the human strain, although elephants are also susceptible to *Mycobacterium bovis*, the cattle strain.

Clinical signs observed in captive elephants include weight loss, difficulty in breathing, abnormal trunk discharges, loss of appetite, and exercise intolerance. In many cases, elephants show no clinical signs until the disease is advanced.

Although TB is typically a chronic disease in captive elephants causing debilitation over a number of years, even infected elephants that appeared clinically normal have died acutely.

Postmortem lesions are mainly found in the lungs and thoracic lymph nodes. *M. tb* may cause small miliary lesions or large granulomas; lesions may be localized (usually in elephants that have died from other causes) or extensive with obliteration of normal lung or lymph node tissues. *M. tb* can also be systemic, involving multiple organ systems (Landolfi *et al.* 2015).

Diagnosis

Types of TB tests

Diagnostic tests for TB can be classified as either direct or indirect. See Table 1. Direct methods that detect TB organisms include culture, acid-fast stain, and nucleic acid amplification techniques such as PCR. Indirect tests are typically immune-based. They detect some change that occurs in response to the presence of the TB bacteria. The intradermal skin test for example measures cellular reactivity against mycobacterial antigens. Serological tests detect antibodies. The interferon gamma assay measures antigen-specific IFN-γ released from the white blood cells of infected humans or animals.

Table 1. Types of tests to diagnose TB

Direct tests	**Indirect tests**
Culture	Intradermal skin test
Acid-fast stain	ELISA
Nucleic acid amplification tests (PCR)	Serology
	Interferon gamma (IFN-γ) assays

No one test is perfect and every test has advantages and disadvantages. Sensitivity and specificity vary. Sensitivity is the the ability of a test to identify true infections and specificity is the ability of a test to truly identify non-infected individuals. The intradermal skin test, used to diagnose TB in cattle has been shown to have poor sensitivity and specificity in elephants and is not recommended (Mikota *et al.* 2001, Lewerin *et al.* 2005).

False-positive and false-negative results can occur with any test. Levels of detection also differ. For example, for TB to be detected using acid-fast smears, a minimum of ~ 1000 organisms/mL must be present. To be detected by culture, ~ 100 organisms/mL must be present. Nucleic acid amplification tests can detect as few as 1-10 organisms, irrespective of whether the organisms are alive or dead. Acid fast smears are not specific for TB – other organisms such as nocardia are also acid-fast as are non-tuberculous mycobacterial species. Species identification requires confirmation by culture or DNA probe.

Culture and PCR

While isolation of mycobacteria provides a definitive diagnosis of TB infection, limitations of culture include the difficulty of obtaining a proper sample, sample contamination, and the need of a biosafety lab with the capability of culturing mycobacteria which may not be readily available everywhere (Mikota *et al.* 2015). *M. tuberculosis* being a slow-growing organism, results take six to eight weeks.

Elephants shed TB intermittently and it may be necessary to collect a large number of samples before a positive culture confirms infection in suspect animals (Moller *et a*l. 2005, Angkawanish *et al*. 2010, Steinmetz *et al*. 2016, Vogelnest *et al*. 2015, Miller *et al*. 2018). A positive culture confirms TB, but a negative culture does not rule it out.

A highly sensitive PCR-RFLP assay developed in Nepal successfully differentiated *M. tuberculosis*, *M. bovis* and *M. avium* in spiked samples of elephant trunk washes. Subsequent evaluation of 22 TB-suspect elephants did not detect any culture positive animals (Smiley-Wilson 2007). The assay was further refined and later used to successfully diagnose *M. tuberculosis* in elephant trunk wash and nasal drip samples (Magnuson *et al.* 2017). TB isolates from Nepal have been further characterized using molecular techniques (Paudel *et al.* 2021, Paudel *et al.* 2019a).

A commercially available PCR kit has been shown to be a sensitive screening test for MTB complex in African elephants, African buffalo, and white rhinos. This and other work from South Africa may have application to Asian elephants (Goosen *et al.* 2022, Goosen et al; 2020a, Goosen *et al.* 2020b).

Indirect tests

Indirect tests do not detect the actual TB organism and therefore do not tell us whether an animal is shedding TB at the time of testing.

The identification of immunodominant antibodies associated with TB in elephants led to the development of a rapid serological test – the ElephantTB STAT-PAK® (Chembio Diagnostics Inc. USA) which is no longer manufactured but has been replaced by the DPP VETTB test. These tests have been used in several studies conducted in range countries. The DPP VetTB test is commercially available from the U.S. The and DPP VetTB® have been shown to detect TB months to years in advance of a positive culture (Paudel *et al.* 2018, Greenwald *et al.* 2009).

Additional serological work has been conducted in India. The Verma-Kumar study (2012) compared Elephant TB STAT-PAK results to four in-house ELISA assays and found 15.9% sero-reactivity on all tests. Rajhans *et al.* (2021) had mixed results testing 15 elephants using a rapid test kit, a BacT/ALERT 3D system, Ziehl-Neelsen staining, and PCR. The aim of the study by Veerasami *et al.* (2018) was to develop a rapid antibody test using the ESAT6-CFP10- fusion protein (also used in the DPP TB test) with the addition of bovine PPD and avium PPD to improve sensitivity and minimize cross-reaction to diagnose TB in cattle. The kit's performance was tested using sera samples from sloth bears and a variety of wildlife species. Sixteen of 17 sloth bears with known TB reacted on the test. The other species which included nine elephants altogether showed a seropositivity of 25%. Further work is needed to evaluate this kit in elephants.

The interferon gamma is another test under investigation to diagnose TB in elephants. It measures antigen-specific gamma interferon released from T- cells. Like serology, it is an indirect test; it has been validated in cattle and humans but not yet in elephants due to the lack of a large enough study population of infected and non-infected elephants (Songthammanuphap et al. 2020, Paudel et al. 2016; Angkawanish et al. 2013).

Selection of tests to diagnose TB in elephants

Diagnosing TB in elephants is difficult. The diagnostic options are limited and the available tests all have advantages and disadvantages. Some tests, like the interferon gamma assay are not commercially available but may be accessed by contacting the lead researcher on the studies listed here. These and other indirect tests can be useful for screening or surveillance and positive or suspect results should be followed by repeat testing, using other tests, or initiating a series of culture testing. If there is a history of TB in captive elephants at the captive-wild interface or in other wildlife species, it may be prudent to segregate suspect elephants while further testing is conducted. Herd history and exposure history are important and should be considered together with lab results. Anyone involved with TB in elephants is advised to read the scientific publications to help guide their decisions.

Guidelines for treatment

Elephants are treated for TB using the same drugs used for humans but generally for a longer period of time (~one year). It is a challenging process as the drugs are bitter and are often refused necessitating rectal administration (which can only be used with certain drugs). Detailed descriptions can be found in the following documents:

Nepal Elephant Tuberculosis Control and Management Action Plan (2011-2015)

https://www.ntnc.org.np/sites/default/files/doc_publication/2018-11/Nepal%20Elephant%20TB%20Control%20and%20Mgt%20Action%20Plan.pdf (currently undergoing revision)

2017 RECOMMENDATIONS FOR THE DIAGNOSIS, TREATMENT AND MANAGEMENT OF TUBERCULOSIS (Mycobacterium tuberculosis) IN ELEPHANTS IN HUMAN CARE
http://www.nasphv.org/Documents/ElephantTB_NASPHV.pdf

GUIDELINES FOR THE CONTROL OF TUBERCULOSIS IN ELEPHANTS 2010
https://www.elephantcare.org/_files/ugd/5c07e7_4c61f2bd45814b12ad4d66595c76add8.pdf

Several pharmacokinetic studies of anti-TB drugs have been conducted in elephants (Maslow *et al.* 2005a, Maslow *et al.* 2005b, Zhu *et al.* 2005, Peloquin *et al.* 2006, Egelund et al 2015). Therapeutic serum drug monitoring if available, can confirm that target levels are reached. A recent case report also suggests that monitoring of IgG may be valuable to assess treatment response (Ishikawa *et al.* 2022). Suga (2021) and colleagues describe intensive treatment of an elephant infected with *Mycobacterium caprae*. Training the elephant for oral and rectal medication is required for prolong antibiotic treatment.

Distribution of TB among captive elephants

Sporadic reports of TB in elephants in Asia were published from as early as 1925 (Narayanan, 1925) but TB in elephants did not receive much attention globally until it became a serious problem in the U.S. in 1996 (Mikota and Maslow, 2011). Following are select reports from Asia:

- Thailand: In 2005-2008, 4 cases were culture confirmed (Angkawanish, *et al.* 2010)
- Thailand: 30% (n=60) reacted to gamma interferon test; 4 of 31 random samples reacted to DPP Vet TB test (Songthammanuphap, *et al.* 2020).
- Nepal: A serosurvey in 2006 showed 13% reactivity on Stat-Pak (n=115) (Mikota *et al.* 2015)
- Nepal: In 2009-2013, 5 cases were culture confirmed (Paudel *et al.* 2014, Paudel *et al.* 2019)
- Nepal: In 2008-2009, a serosurvey revealed 21.56% reactivity on Stat-Pak (Thapa *et al.* 2021)
- India: In 2008, 45 of 300 elephants reacted on Stat-Pak (Abraham *et al.* 2008)
- Malaysia: In 2012, 20.4% were found seropositive on Stat-Pak (n=63); three trunk wash samples were *M. tb* PCR positive (Ong *et al.* 2013)
- Laos: A serosurvey in 2012, 26% reacted to test using Stat-Pak (n=80) (Lassausaie *et al.* 2015)

Distribution of TB in wild populations

The first reported case of TB in a wild elephant was in an African elephant that had been under human care and was subsequently released in Kenya (Obanda et al, 2013). The first case of TB in

a wild Asian elephant was reported from Sri Lanka (Perera *et al.* 2015). Subsequently, TB was found in a single wild elephant at the Rajiv Gandhi National Park, India (Chandranaik *et al.* 2017) and in three wild elephants at the Wayanad Wildlife Sanctuary in southern India (Zachariah *et al.* 2017).

While close contact with humans can explain the occurrence of TB in captive elephants, the epidemiology of TB among wild elephants is not clear. In the cases reported thus far in wild elephants in India and Sri Lanka, there were no known releases of captive elephants and no or negligible interactions with captive elephants. Though the exact mechanism is not known, M. tuberculosis is a reverse zoonoses spilling over into wild elephant populations.

Habitat encroachment and human-elephant conflict brings wild elephants into closer contact with humans, providing increased opportunities for pathogen transmission. Captive elephants infected with TB could spread the disease to wild elephants while sharing grazing lands or during activities such as tourist safaris or patrolling, or even while mating with wild counterparts. Fomites are also a possibility. If this disease becomes established in the wild, other susceptible species will also be at risk. To make matters worse, reports of wild elephants scavenging on garbage dumps are also on the rise.

Mitigation and management (minimizing spread)

TB is a zoonotic One-Health disease (Abraham and Pillai 2016, Paudel *et al.* 2019b, Paudel *et al.* 2019c, Paudel and Sreevatsan 2020, Yakuba et al 2016, Sookaromdee and Wijwanitkit 2020, Rajbhandari *et al.* 2022) and the chronic nature affects the welfare of affected animals (Szydlowski 2022).

It is important to conduct surveillance of all species involved. This may include elephants, humans, and domestic species such as cattle. Only a few studies of captive elephants have also tested humans in together, but this is essential to break the transmission cycle.

TB can be a disease with economic effects. A diagnosis of TB can have serious implications for individuals or facilities that generate income from captive elephants. They may try to hide the fact that they have TB or may actively oppose any regulations that are perceived as a threat to their business. Some private owners may sell or transfer elephants with TB to other unsuspecting owners. A TB diagnosis may affect insurance reimbursement for deceased elephants. The worst-case scenario would be for infected captive elephants in Asia to be released to the wild. Because of these issues, TB in elephants is probably under-reported.

To minimize transmission to the wild, TB must be managed at the captive-wild interface. We first need to identify key areas where captive elephants intermingle with wild elephants during tourism, patrolling, or grazing, and institute regular surveillance.

Our first task is to identify the scope of the problem. To accomplish this, a designated AsESG representative (or other point person) in each range country could help to:

1. Identify the agency or agencies in charge of elephants
2. Identify (and map) locations where there is known intermingling between captive and wild elephants.
3. Assess whether TB has been found in either captive or wild elephants in these locations (possibly through a simple online survey).
4. Determine what level of surveillance may already be in place (e.g. do wildlife officials always perform postmortem examinations of wild elephants? How thorough are the examinations? Are captive elephants necropsied? Who receives reports? Is pathology training needed?) Ideally, a comprehensive post-mortem examination should be performed on all captive and wild elephants that die including submitting key tissues (lung and lymph nodes) to check for TB even if no gross lesions are present.
5. Once potential hotspots are identified we can assist with specific recommendations. Needs may differ, so also the available resources to carry out surveillance.
6. Routine testing of humans who work closely with elephants at captive facilities should be encouraged. Elephants managed by individuals that are TB positive should be targeted for testing and contact tracing initiated.

If need be, the authorities should plan to relocate captive elephant camps that are not critical to wildlife management out of the protected areas. The needless mushrooming of captive elephant camps within the protected areas should be stopped. The practice of mixing confiscated private elephants with forest camp elephants should be discouraged. If relocation to forest areas is unavoidable, the private elephants brought up in urban and suburban settings should be ideally settled in areas where wild elephants do not frequent. If this is not practicable, as a last resort, they should be quarantined and screened for tuberculosis before being taken to the site.

TB has been a problem among captive elephants in Nepal. Nepal has had a surveillance plan in place for several years. However, when testing of the captive population lapsed there was an upsurge in cases (unpublished). The Nepal Elephant Tuberculosis Control and Management Action Plan (2011-2015) was endorsed by the Ministry of Forestry as part of Nepal's Elephant Action Plan. The Plan needs to be updated. Nonetheless it can serve as a model for other countries.

Segregation or treatment of infected elephants can minimize transmission to the wild. Treatment is challenging and expensive (and beyond the scope of this document) but has been carried out successfully in a number of cases in Nepal. Segregation means isolation of known infected elephants (diagnosed by culture) or highly suspect elephants determined by history and/or serology. They should not be allowed to graze, patrol, or used for tourism in areas where contact with wild elephants is possible. The health of humans working with infected elephants also needs to be considered.

Other Recommendations

1. Contact wildlife departments in the 13 range countries to inform them about AsESG concern for these diseases of concern. Communicate the importance of disease surveillance and comprehensive postmortem examination of wild and captive elephants. Determine their needs. Let them know about these resources:

McManamon, R. and Terrell, S.P. (2011) Practical application of general principles: elephant postmortem examination. *Tuberculosis in Elephants: Science, Myths, and Beyond, April 5–6, 2011*. USDA, APHIS Center for Animal Welfare, Kansas City, Missouri. Available at: https://www.aphis.usda.gov/animal_welfare/downloads/elephant/Postmortem%20Exam%20Procedures.pdf

Elephant Pathology Lecture Series (course conducted in Myanmar):

http://elephantcare.org/resources/pathology/. These materials are freely available to be used for other courses.

2. Establish a central repository for PM reports from range countries.

3. Post information related to EEHV and TB on the AsESG website.

4. Consider establishing country reps responsible for monitoring and reporting cases of EEHV and TB to the AsESG Chair.

5. Develop and send a survey to the EEHV labs to determine how many cases they have diagnosed (make sure to ask how many of these have been reported in the literature, to avoid double counting).

Literature cited:

Note: a comprehensive list of elephant TB publications can be found at www.eleophantcare.org

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Abraham, D. and J. Davis (2008). Revised trunk wash collection procedure for captive elephants in a range country setting. *Gajah.* 28: 53-54

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DNPWC (2011): *Nepal Elephant Tuberculosis Control and Management Action on Plan (2011-2015).* Government of Nepal, Ministry of Forests and Soil Conservation, Department of National Parks and Wildlife Conservation, Kathmandu, Nepal. 39 pages

Egelund, E.F., R. Isaza, A.P. Brock, A. Alsultan, G. An, and C.A. Peloquin (2015). Population pharmacokinetics of rifampin in the treatment of *Mycobacterium tuberculosis* in Asian elephants. *Journal of Veterinary Pharmacology and Therapeutics.* 38: 137-143

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Narayanan, R.S. (1925). A case of tuberculosis in an elephant. *Journal of Comparative Pathology*. 38: 96–97

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Paudel, S., C., Nakajima S.K. Mikota, K.P., Gairhe, B. Maharjan, S. Subedi, A. Poudel, M. Sashika, M., M. Shimozuru, Y. Suzuki and T. Tsubota (2019). Mixed *Mycobacterium tuberculosis* lineage infection in 2 elephants, Nepal. *Emerg Infect Dis.* 25: 1031-1032

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complex in elephants through a One Health approach: a systematic review. *BMC Vet Res*. 18(1):262

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Sookaromdee, P. and V. Wiwanitkit (2020). Zoonotic possibility of tuberculosis from domestic elephants: a case assessment from Thailand. *Egyptian Journal of Chest Diseases and Tuberculosis.* 69: 447-448

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Veerasami, M., K. Venkataraman, C. Karuppannan, A.A. Shanmugam, M.C. Prudhvi, T. Holder, P. Rathnagiri, K., Arunmozhivarman, G.D. Raj, M. Vordermeier, B. Mohana Subramanian (2018). Point of care Tuberculosis sero-diagnosis kit for wild animals: combination of proteins for improving the diagnostic sensitivity and specificity. *Indian Journal of Microbiology*. 58 (1): 1-12

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Zachariah A., J.C. Zong, S.Y. Long, E.M. Latimer, S.Y. Heaggans, L.K. Richman, and G.S. Hayward (2013). Fatal herpesvirus hemorrhagic disease in wild and orphan Asian elephants in Southern India. *Journal of Wildlife Diseases.* 49(2):381–393

Zachariah, A., J. Pandiyan, G. K. Madhavilatha, S. Mundayoor, B. Chandramohan, P. K. Sajesh, S. Santhosh and S. K. Mikota (2017). *Mycobacterium tuberculosis* in Wild Asian Elephants, Southern India. *Emerging Infectious Diseases*. 23(3): 504-506

Zhu, M., J.N. Maslow, S.K. Mikota, R. Isaza, F. Dunker, H. Riddle, and C.A. Peloquin, (2005). Population pharmacokinetics of pyrazinamide in elephants. *J. Vet. Pharmacol. Ther.* 28: 403-409

Annexure 1. EEHV Literature Summary (as on October 2020)

Reported country	Captive/Wild/Both	Age class	Cause (agent)
Thailand	Captive. Serosurvey of 994 elephants; 420 (42.3%) were sero-positive	< 11 years to > 50 years	EEHV 1-A
Angkawanish, T., M. Nielen, H. Vernooij, J. L. Brown, P. J. S. van Kooten, P. B. van den Doel, W. Schaftenaar, K. Na Lampang and V. Rutten (2019). "Evidence of high EEHV antibody seroprevalence and spatial variation among captive Asian elephants (*Elephas maximus*) in Thailand." *Virology Journal*. 16(1): 33.			
Thailand	Captive: 58 confirmed cases 2006-2018. Mortality 68.97%.	Median age 29 months	EEHV
Boonprasert, K., V. Punyapornwithaya, P. Tankaew, T. Angkawanish, S. Sriphiboon, C. Titharam, J. L. Brown and C. Somgird (2019). "Survival analysis of confirmed elephant endotheliotropic herpes virus cases in Thailand from 2006 - 2018." *PloS ONE* 14(7): e0219288.			
India	13 new cases between 2013-2017: 8 wild, 3 camp-raised orphans, 2 captive-born calves		EEHV 1-A
Zachariah, A., P. K. Sajesh, S. Santhosh, C. Bathrachalam, M. Megha, J. Pandiyan, M. Jishnu, R. S. Kobragade, S. Y. Long, J. C. Zong, E. M. Latimer, S. Y. Heaggans and G. S. Hayward (2018). "Extended genotypic evaluation and comparison of twenty-two cases of lethal EEHV1 haemorrhagic disease in wild and captive Asian elephants in India." *PloS ONE* 13(8): e0202438.			
Thailand	captive / wild	7 month old captive-born; 3 month old wild-born	EEHV 1-A; EEHV 4 / Clostridium
Boonsri, K., C. Somgird, P. Noinafai, K. Pringproa, T. Janyamethakul, T. Angkawanish, J. L. Brown, P. Tankaew, S. Srivorakul and C. Thitaram (2018). "Elephant Endotheliotropic herpesvirus associated with *Clostridium perfringens* infection in two Asian elephant (*Elephas maximus*) calves." *Journal of Zoo and Wildlife Medicine*. 49(1): 178-182.			
India	captive	Calf	EEHV 1-A
Barman, N. N., B. Choudhury, V. Kumar, M. Koul, S. M. Gogoi, E. Khatoon, A. Chakroborty, P. Basumatary, B. Barua, T. Rahman, S. K. Das and S. Kumar (2017). "Incidence of elephant endotheliotropic herpesvirus in Asian elephants in India." *Veterinary Microbiology.* 208: 159-163.			
India	3 wild (rescued) calves PCR positive of 22 calves; 3 of 267 adult captive elephant PCR positive	Calf	EEHV 1-A
Mahato, G., K. K. Sarma, D. C. Pathak, N. N. Barman, P. Gogoi, M. Dutta and P. Basumatary (2019). "Endotheliotropic herpesvirus infection in Asian elephants (*Elephas maximus*) of Assam, India." *Vet World* 12(11): 1790-1796.			

Thailand	captive: 56 elephants tested using DNA primers; one sample was positive	Calf; 3 years old asymptomatic	EEHV 4
Bhusri, B., P. Suksai, C. Mongkolphan, E. Tiyanun, P. Ratanakorn, K. Chaichoun and L. Sariya (2017). "Detection of elephant endotheliotropic herpesvirus 4 in captive Asian elephants (*Elephas maximus*) in Thailand." *Thai Journal of Veterinary Medicine* 47(1): 97-102.			
Asia - EEHV Management Guidelines	NA	NA	EEHV
Luz, S. and L. Howard (2017). Guidelines for Management Elephant Endotheliotropic Herpesvirus in Asia 2**nd** edition, Wildlife Reserves Singapore Group: 19.			
Thailand	captive	Calf 3 years old (successful treatment)	EEHV 1-A
Sripiboon, S., T. Angkawanish, K. Boonprasert, P. Sombutputorn, W. Langkaphin, W. Ditcham and K. Warren (2017). "Successful treatment of a clinical elephant Endotheliotropic herpesvirus infection: The dynamics of viral load, genotype analysis, and treatment with acyclovir." *Journal of Zoo and Wildlife Medicine* 48(4): 1254-1259.			
Thailand	24 cases between 2006-2014; 18 of 24 PCR positive; all captive-born except one case	1-9 years	EEHV 1-A - 13 cases; EEHV 1B - 2 cases; EEHV 4 - 3 cases
Sripiboon, S., B. Jackson, W. Ditcham, C. Holyoake, I. Robertson, C. Thitaram, P. Tankaew, P. Letwatcharasarakul and K. Warren (2016). "Molecular characterisation and genetic variation of Elephant Endotheliotropic Herpesvirus infection in captive young Asian elephants in Thailand." *Infection Genetics and Evolution*. 44: 487-494.			
Thailand	8 cases 2007-2013; 6 deceased and 2 surviving	1 year 6 months - 3 years	EEHV 1-A - 5 cases; EEHV 1B - 1 case; EEHV 4 - 2 cases
Lertwatcharasarakul, P., P. Sanyathitiseree, N. Thongtip, P. Charoenphan, B. Boonyasart, N. Maneewan and T. Songserm (2015). "Genetic variant of elephant endotheliotropic herpesvirus detected from captive Asian elephants (*Elephas maximus*) in Thailand from 2007 to 2013." *Thai Journal of Veterinary Medicine.* 45(1): 73-79.			
Thailand	captive	all age groups	subclinical infection detected in5.5% of 2362 elephants
Sripiboon, S., W. Ditcham, R. Vaughan-Higgins, B. Jackson, I. Robertson, C. Thitaram, T. Angkawanish, S. Phatthanakunanan, P. Lertwatcharasarakul and K. Warren (2020). "Subclinical infection of captive Asian elephants (*Elephas maximus*) in Thailand with elephant endotheliotropic herpesvirus." *Archives of Virology*. 165(2): 397-401.			
India	captive/ wild	calf (2 cases)	EEHV
Elephant Endotheliotropic Herpes Virus (EEHV) caused fatality in wild and captive orphan Asian elephant calves in North-eastern India- Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati- 781022			
Laos	captive / wild	Calf 2.5 years	EEHV 1-A

	Bouchard, B., B. Xaymountry, N. Thongtip, P. Lertwatcharasarakul and W. Wajjwalku (2014). "First reported case of elephant endotheliotropic herpes virus infection in Laos." *Journal of Zoo and Wildlife Medicine.* 45(3): 704-707.		
Nepal	captive	15 calves; 2 adults	No disease - PCR of all samples (blood and conjunctival swabs) was negative
	Pandit, A., I. P. Dhakal, K. Gairhe, H. B. Rana and D. Karmacharya (2014). "Surveillance of the Elephant Endotheliotropic Herpesvirus (Eehv) in Chitwan district, Nepal." *International Journal of Recent Scientific Research.* 5(10).		
India	captive: 46 elephants	3 month - 75 years	No disease - subclinical infections of EEHV 1, EEHV 3/4, and EEHV 5 detected
	Stanton, J. J., S. A. Nofs, A. Zachariah, N. Kalaivannan and P. D. Ling (2014). "Detection of elephant endotheliotropic herpesvirus infection among healthy Asian elephants (*Elephas maximus*) in South India." *Journal of Wildlife Diseases.* 50(2): 279-287.		
Thailand	captive	two calves 2 years and 3 years of age	EEHV
	Sripiboon, S., P. Tankaew, G. Lungka and C. Thitaram (2013). "The occurrence of elephant endotheliotropic herpesvirus in captive Asian elephants (*Elephas maximus*): first case of EEHV4 in Asia." *Journal of Zoo and Wildlife Medicine*. 44(1): 100-104.		
India	15 cases: 7 wild 2 captive-born, 6 wild born orphans in captivity	juvenile - subadult	EEHV 1-A; EEHV 1B
	Zachariah, A., J. C. Zong, S. Y. Long, E. M. Latimer, S. Y. Heaggans, L. K. Richman and G. S. Hayward (2013). "Fatal herpesvirus haemorrhagic disease in wild and orphan Asian elephants in southern India." *Journal of Wildlife Diseases*. 49(2): 381-393.		
Cambodia	wild	~ 3 year-old females	EEHV
	Reid, C. E., T. B. Hildebrandt, N. Marx, M. Hunt, N. Thy, J. M. Reynes, W. Schaftenaar and J. Fickel (2006). "Endotheliotropic elephant herpes virus (EEHV) infection. The first PCR-confirmed fatal case in Asia." *Veterinary Quarterly*. 28(2): 61-64.		
Myanmar	captive	3 elephant cases: 20 mo; 22 mo; 16 mo	EEHV 1-A
	Oo, Z. M., Y. H. Aung, T. T. Aung, N. San, Z. M. Tun, G. S. Hayward and A. Zachariah (2020). "Elephant Endotheliotropic Herpesvirus Haemorrhagic Disease in Asian Elephant Calves in Logging Camps, Myanmar." *Emerging Infectious Diseases.* 26(1): 63-69.		
Sri Lanka	Wild	1 elephant case: 4 year old female	EEHV 1-A
	Perera, B. V. P., De Silva, D., Wijesundara, K., Sripiboon, S., & Latimer, E. (2018). The first confirmed case of elephant endotheliotropic herpesvirus (EEHV) infection in Sri Lanka. Paper presented at the 70th Annual Convention of Sri Lanka Veterinary Association.		

Annexure 2. Country-wise tuberculosis literature summary (as of December 2022)

Country	Captive/ Wild/Both	Age class	Cause (agent)
Nepal	Captive	adults (8 cases)	*M. tuberculosis*
	Paudel, S., S. K. Mikota, J. Thapa, K. P. Lyaschenko, K. P. Gairhe, I. P. Dhakal, N. Subedi, B. Maharjan, S. Subedi, G. E. Kaufman and T. Tsubota (2018). "Serodiagnosis of elephant tuberculosis: a useful tool for early identification of infected elephants at the captive-wild interface." *European Journal of Wildlife Research*. 64: 70.		
India	Wild	adults (3 cases)	*M. tuberculosis*
	Zachariah, A., J. Pandiyan, G. K. Madhavilatha, S. Mundayoor, B. Chandramohan, P. K. Sajesh, S. Santhosh and S. K. Mikota (2017). "Mycobacterium tuberculosis in Wild Asian Elephants, Southern India." *Emerging Infectious Diseases.* 23(3): 504-506.		
Sri Lanka	Wild	adult (1 case)	*M. tuberculosis*
	Perera et al 2014: "First confirmed case of fatal tuberculosis in a wild Sri Lankan elephant." *Gajah* 41: 28-31.		
Nepal	Captive	adults (3 cases)	*M. tuberculosis*
	Paudel, S., S. K. Mikota, C. Nakajima, K. P. Gairhe, B. Maharjan, J. Thapa, A. Poudel, M. Shimozuru, Y. Suzuki and T. Tsubota (2014). "Molecular characterization of Mycobacterium tuberculosis isolates from elephants of Nepal." *Tuberculosis (Edinb)* 94(3): 287-292.		
Thailand	Captive	adults	*M. tuberculosis*
	Angkawanish, T., W. Wajjwalku, A. Sirimalaisuwan, S. Mahasawangkul, T. Kaewsakhorn, K. Boonsri and V. P. M. G. Rutten (2010). "*Mycobacterium tuberculosis* infection of domesticated Asian elephants, Thailand." *Emerging Infectious Diseases.* 16(12): 1949-1951.		
Nepal	Captive	One Health approach and TB program in Nepal - Review	*M. tuberculosis*
	Mikota, S. K., G. E. Kaufman, N. Subedi and I. P. Dhakal (2016). *Mycobacterium tuberculosis* in Elephants in Asia - Taking a One Health Approach. "One Health Case Studies: Addressing Complex Problems in a Changing World." S. Cork, Hall, D.C., and Liljebelke, K. Sheffield, U.K. 5m Publishing: 54-64.		
Nepal	Captive	One Health approach and TB program in Nepal - Review	*M. tuberculosis*
	Rajbhandari R.M., J. de la Fuente, D. Karmacharya, S. Mathema, B. Maharjan, S.M. Dixit, N. Shrestha, J. Queirós, C. Gortázar, P.C. Alves (2022). Understanding Mycobacterium tuberculosis complex in elephants through a One Health approach: a systematic review. *BMC Vet Res*. 18(1):262.		

General (letter)	Captive/wild	NA	*M. tuberculosis*
Paudel, S., S. K. Mikota and T. Tsubota (2019). "Tuberculosis threat in Asian elephants." *Science* 363(6425): 356.			
Nepal	Captive/wild	2002-2015 13 elephants died of suspected or confirmed:	*M. tuberculosis*
Paudel, S., S. K. Mikota, J. Thapa, K. P. Lyaschenko, K. P. Gairhe, I. P. Dhakal, N. Subedi, B. Maharjan, S. Subedi, G. E. Kaufman and T. Tsubota (2018). "Serodiagnosis of elephant tuberculosis: a useful tool for early identification of infected elephants at the captive-wild interface." *European Journal of Wildlife Research* 64: 70.			
Nepal	Captive/wild	development of assay	*M. tuberculosis*
Paudel, S., M. A. Villanueva, S. K. Mikota, C. Nakajima, K. P. Gairhe, S. Subedi, N. Rayamajhi, M. Sashika, M. Shimozuru, T. Matsuba, Y. Suzuki and T. Tsubota (2016). "Development and evaluation of an interferon-γ release assay in Asian elephants (*Elephas maximus*)." *Journal of Veterinary Medical Science* 78(7): 1117-1121			
Nepal	Captive		Serosurvey in 2006: 13% reactive on Stat-Pak n=115
Mikota, S. K., K. Gairhe, K. Giri, K. Hamilton, M. Miller, S. Paudel, K. Lyashchenko, R. S. Larsen, J. B. Payeur, W. R. Waters, R. Greenwald, G. Dumonceaux and B. Vincent (2015). "Tuberculosis surveillance of elephants (*Elephas maximus*) in Nepal at the captive-wild interface." *European Journal of Wildlife Research*. 61: 221-229.			
Thailand	Captive/wild	Development of assay	*M. tuberculosis*
Angkawanish, T., D. Morar, P. van Kooten, I. Bontekoning, J. Schreuder, M. Maas, W. Wajjwalku, A. Sirimalaisuwan, A. Michel, E. Tijhaar and V. Rutten (2013). "The elephant interferon gamma assay: a contribution to diagnosis of tuberculosis in elephants." *Transboundary and Emerging Diseases*. 60 Suppl 1: 53-59.			
India	Captive		Serosurvey 45 of 300 elephants reactive on Stat-Pak
Abraham D, Cheeran JV, Sukumar R, Mikota SK, Rao S, Ganguly S, *et al.* "Health assessment of captive Asian elephants in India with special reference to tuberculosis." Report to Project Elephant. New Delhi: Ministry of Environment and Forests, Government of India; 2008.			
Malaysia	Captive		Serosurvey 20.4% reactive on Stat-Pak (N- 63); 3 elephants PCR positive
Ong, B. L., Y. F. Ngeow, M. F. Razak, Y. Yakuba, Z. Zakaria, A. R. Mutalib, L. Hassan, H. F. Ng and K. Versahib (2013). "Tuberculosis in captive Asian elephants (*Elephas maximus*) in Peninsular Malaysia." *Epidemiology and Infection.* (141): 1481-1487.			
Laos	Captive		Serosurvey 36% reactive on Stat-Pak; n=80
Lassausaie, J., A. Bret, X. Bouapao, V. Chanthavong, J. Castonguay-Vanier, F. Quet, S. K. Mikota, C. Theoret, Y. Buisson and B. Bouchard (2015). "Tuberculosis in Laos, who is at risk: the mahouts or their elephants?" *Epidemiology and Infection*. 143(5): 922-931.			

Annexure 3. Contacts of select EEHV Testing Labs in Asia

(From eehv.org website, accessed 10 Oct 2020; updated)

Each lab should be contacted to make sure the information is current.

1. ICAR-Indian Veterinary Research Institute (IVRI)

Contact Information

Joint Director
Phone: +91 0581-2302188
Email: jdcadrad@ivri.res.in

Shipping address

Centre for Animal Disease Research and Diagnosis (CADRAD)
ICAR-IVRI
Izatnagar- 243122
Bareilly
Uttar Pradesh, India

Available Tests

TB- Direct sputum examination, Animal testing, Culture, PCR based diagnosis

2. EEHV Testing at the Centre for Wildlife Studies, Kerala

Contact Information

Dr. George Chandy
Special Officer Mob: +91 99467 34408, +91 8304073367
E mail: chandy@kvasu.ac.in

Shipping address

Centre for Wildlife Studies
Kerala Veterinary and Animal Science University
Pookode, Lakkidi (P.O.)
Wayanad (Dist), Kerala (State). India.673576
+ 91(0)4936 256 381(Office)
+ 91(0) 495 229 8261(Home)

+ 91(0)994 706 8500(cell)
E-mail: zacharun@gmail.com

3. NABL- Accredited laboratory for EEHV and designated laboratory for animal TB in Kerala

Contact Information

1. Chief Disease Investigation Officer Phone +91 472 2840252, Email: cdio.ahd@kerala.gov.in

Dr Ajithkumar G S, Veterinary Surgeon SIAD Mob: 9497268553, 79007768042, Email: ajithnairgs@gmail.com

Shipping address

State Institute of Animal Diseases (SIAD) – Centre for Wildlife Sciences
Animal Husbandry Department, Government of Kerala,
Pacha Palode PO
Thiruvananthapuram Dt. Kerala, India Pin 695562

2. Advanced Disease Monitoring and Diagnostic Facility (AdMAC), Guwahati, Assam

Contact information

Dr. N.N. Barman
Phone: +91 94355 58788
Email: nnbarman@gmail.com

Shipping address

Office of the Core Lab. AdMAC.
College of Veterinary Science,
AAU, Khanapara,
Guwahati – 781022. Assam, India

Available Tests

PCR, sequencing

3. Kasetsart University Elephant Herpesvirus Lab

Contact Information

Dr Supaphen Sripiboon (Amm)
TEL. +66 819880525
E-mail: ssripiboon@gmail.com

Available Tests:

- EEHV confirmation using PCR, sequencing and genotyping
- Quantitative probe real-time PCR for EEHV1, EEHV3/4, EEHV5 infection
- In-house ELISA test for EEHV1 infection

Sample types

Whole blood, fresh tissue samples, swab samples, trunk wash

*NOTE: please indicate name and contact details of person who sending the samples, elephant name, sex, age, brief history and clinical signs, and please contact us before sending the samples.

Shipping address

Faculty of Veterinary Medicine, Kasetsart University,
(Kamphaengsaen campus), Kamphaengsaen
Nakorn Prathom 73140 THAILAND

4. Faculty of Veterinary Medicine, Chiang Mai University, Thailand

Contact Information

Chatchote Thitaram, DVM, PhD, Dipl. TBT
E-mail: chatchote.thitaram@cmu.ac.th

Shipping address

Center of Excellence in Elephant Research and Education
Faculty of Veterinary Medicine, Chiang Mai University
MaeHiae, Muang, Chiang Mai 50100
THAILAND
Tel: +66-53-948015, 948097
Fax: +66-53-274710

Available Tests

PCR, sequencing, genotyping

5. National Trust for Nature Conservation, Biodiversity Conservation Center Molecular Laboratory

Contact Information

Dr. Amir Sadaula
+9779855084907
E-mail: naturalamir@gmail.com

Tests

EEHV confirmation using PCR

Sample Types

Whole blood, fresh tissue samples, swab samples, trunk wash

6. Veterinary Research and Development Center (Lower Northeastern Region), Surin Province

Contact Information

Dr Bopit Puyati
E-mail: bpuyati@gmail.com

Available test

PCR, Sequencing and Probe real-time PCR

7. Elephant Hospital, Thai Elephant Conservation Center, Lampang Province

Contact Information

Dr Taweepoke Angkawanish
E-mail: taweepoke@gmail.com

Available test

PCR

8. Diagnostic Lab, Wildlife Reserves Singapore

Contact Information

Dr. Chia-Da Hsu, BVM, M.Sc., Dipl. ACCM
Senior Veterinary Pathologist
E-mail: chiada.hsu@wrs.com.sg

Shipping address

Conservation, Research and Veterinary Services
Wildlife Reserves Singapore
80 Mandai Lake Road
Singapore 729826
Tel: +65-6360-2233

Available Tests

- Real-time PCR- EEHV1, 3/4, 5
- Conventional PCR (and DNA sequencing)- EEHV1a, 1b, 3/4, 5
- Whole blood (min 1ml), blood flocked swab, FTA card (tissue/blood), frozen/fresh tissue samples, swab samples, trunk wash, extracted DNA samples
- Histopathology: Formalin tissue samples, tissue blocks, unstained slides, H&E-stained slides

Shipping for International Samples

Tissue and blood samples require CITES import/export permits (except for histopathology slides), which must be applied before shipping. It may take months to arrange the permits. Please contact Dr. Chia-Da to start the process.

Please provide following detail of the samples:

1. Name and address
2. Name/ID of the elephant
3. Location of the elephant (zoo, camp or wild)
4. Species
5. Sex
6. Age
7. Captive or wild-born

Please contact us before sending samples to discuss the cost of sample testing. Financial support may be available in some cases.

9. Faculty of Veterinary Science, Syiah Kuala University, Banda Aceh, Indonesia

Contact Information

Dr Muhammad Hambal
hambal.m@unsyiah.ac.id

Available

Conventional and real time PCR

10. Satwa Duta Medical Laboratory for Animal Health

Contact Information

Dr. Adin Priadi
E-mail: priadiadin@gmail.com

Available Tests:

Conventional PCR

Annexure 4. Diagnosis and treatment guidelines for treating elephants for EEHV in Asia

(refer to the PDF attachment)