



# ENDOTHELIOTROPIC ELEPHANT HERPES VIRUS INFECTIONS IN ASIAN ELEPHANTS OF INDIA

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# Incidence reporting

- No historic references in old treatise or camp records
- First hemorrhagic report with lesions of EEHV was reported but not confirmed in Mudumalai elephant camp, Tamilnadu India
- Confirmed Index case was a 9 year old captive born male 'Sunny' in Muthanga elephant camp, Kerala
- Since then 32 cases were reported across India
- All cases were sporadic except for mortality of two calves in contact.
- Primarily affecting young animals
- Sex wise bias not observed

## Regional distribution of cases

- 28 cases were reported in Southern India
- 2 cases from orphan camp in Assam, North – east India
- 2 cases from an elephant camp from Tadoba Tiger Reserve in Maharashtra, Central India
- Four where captive born, eight was orphan calves raised in camps and 18 from free ranging wild elephant populations

# EEHV in captive Elephants-14 death,8 captive orphan calves and Six captive born calves





# EEHV in free ranging elephants-18 cases





# Conducting Post-Mortem in Free Ranging and Captive cases



# Methodology

- Conducting systematic post-mortem in all elephant deaths.
- Collection of appropriate samples and gross lesions recorded.
- Histo-pathological examinations for inclusion bodies.
- PCR amplification and sequencing.
- Interpretation of epidemiological relations .

# CLINICAL AND PATHOLOGICAL LESIONS

## CLINICAL SIGNS

- Per acute in onset
- Pyrexia, Anorexia  
,Dyspepsia and dullness
- Facial edema, Cyanosis of  
the tongue

## PATHOLOGICAL LESIONS

- Extensive  
hemorrhagic lesions
- Petechiae of pericardium  
& endocardium
- pericardial effusion
- Petechiae of peritoneum
- Hepatomegaly
- Intestinal hemorrhage and  
ulceration



# Gross pathology-Facial edema

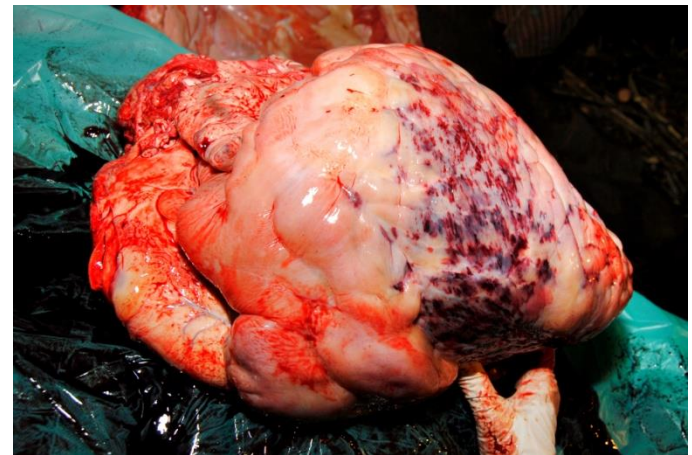
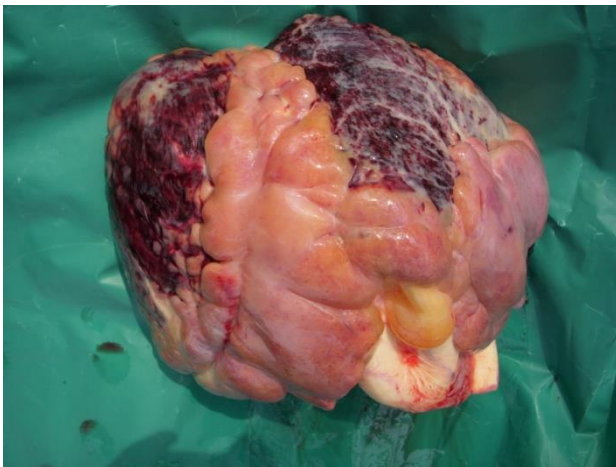


# Cyanosis of the tongue



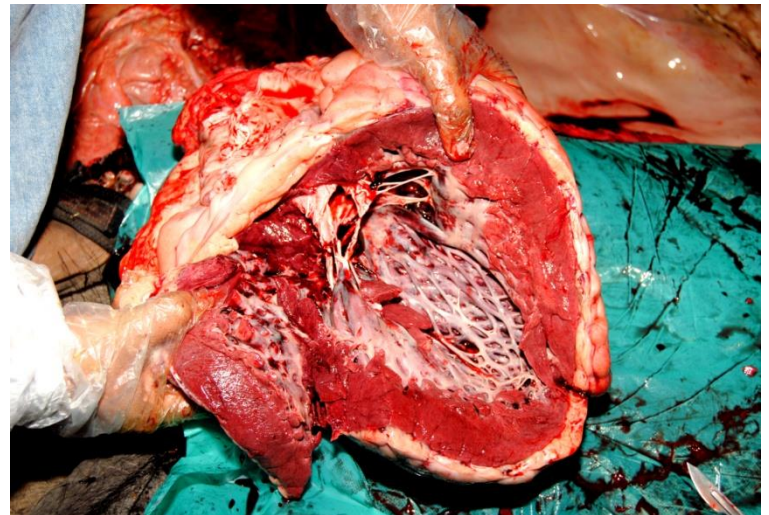
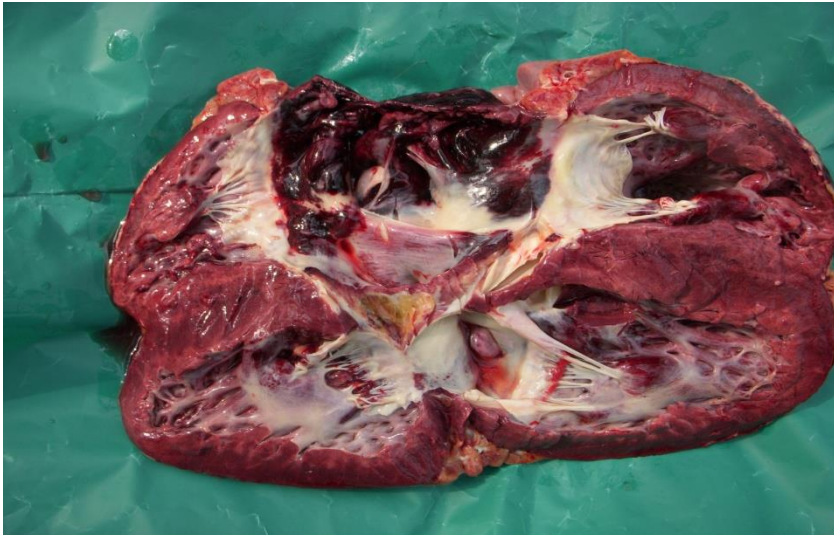


# Gross pathological lesions



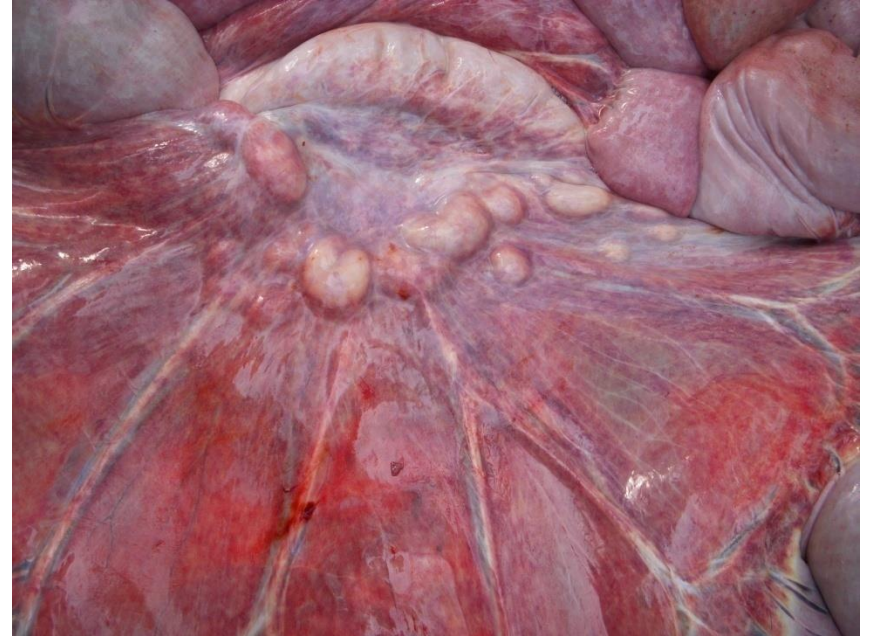


# Gross pathological lesions

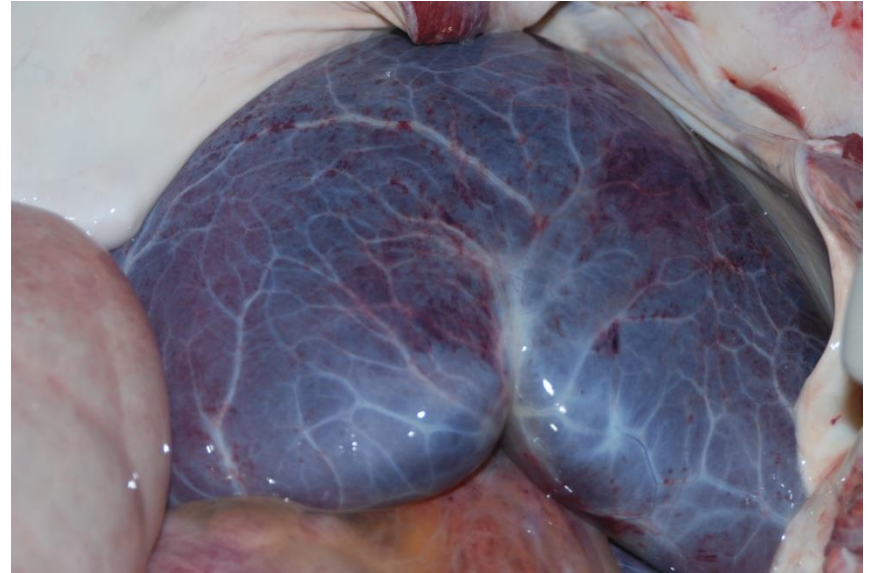
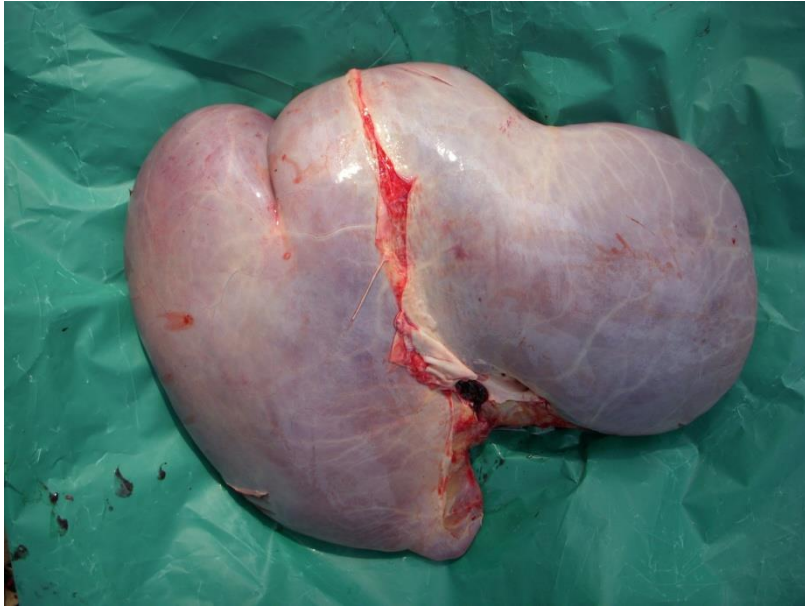




# Gross pathological lesions

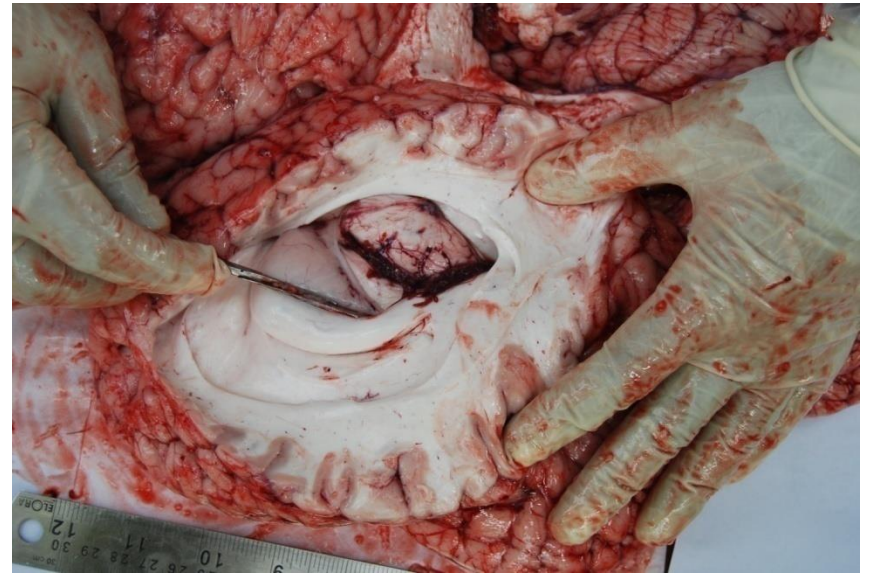


# Gross pathological lesions



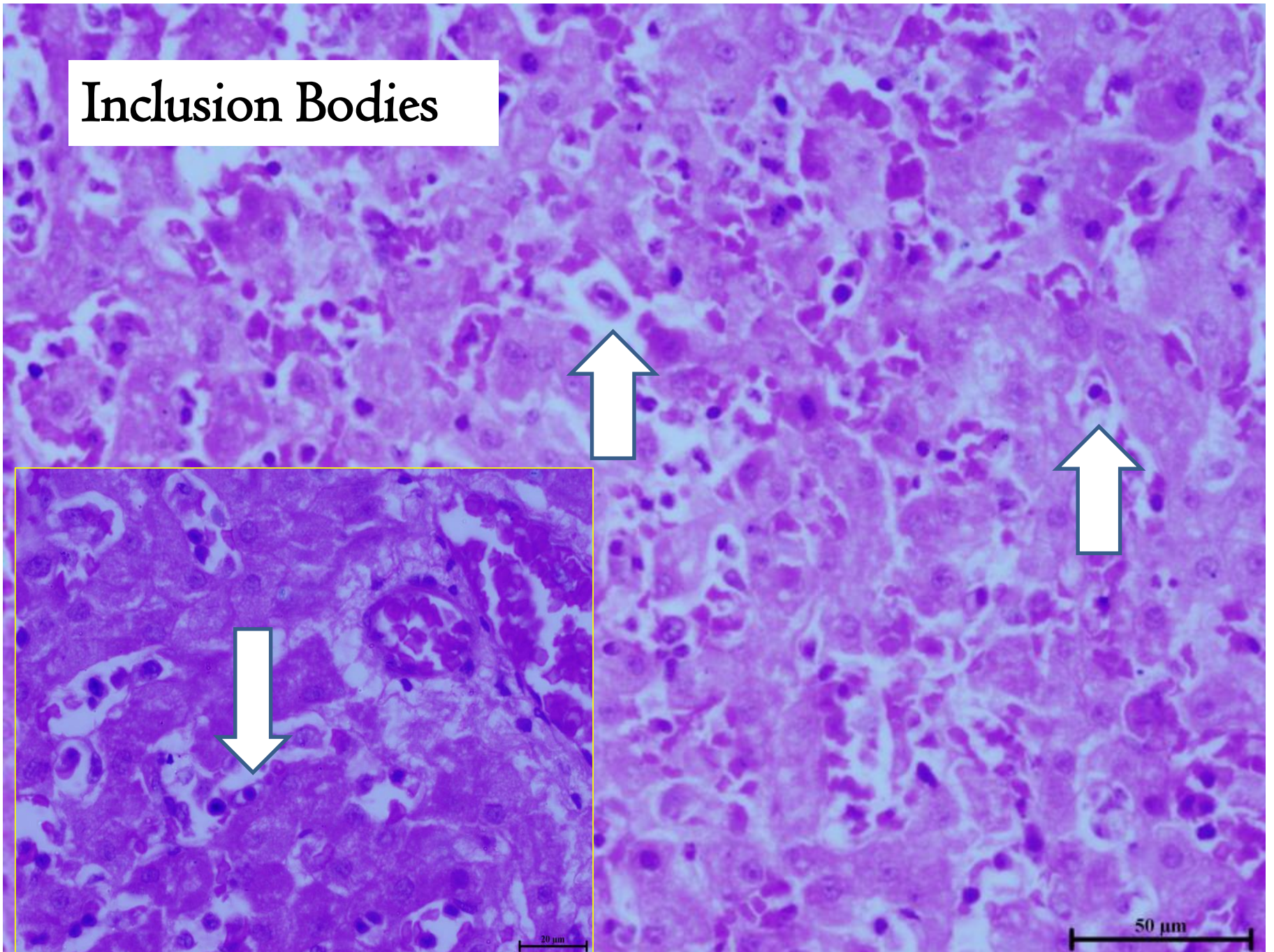


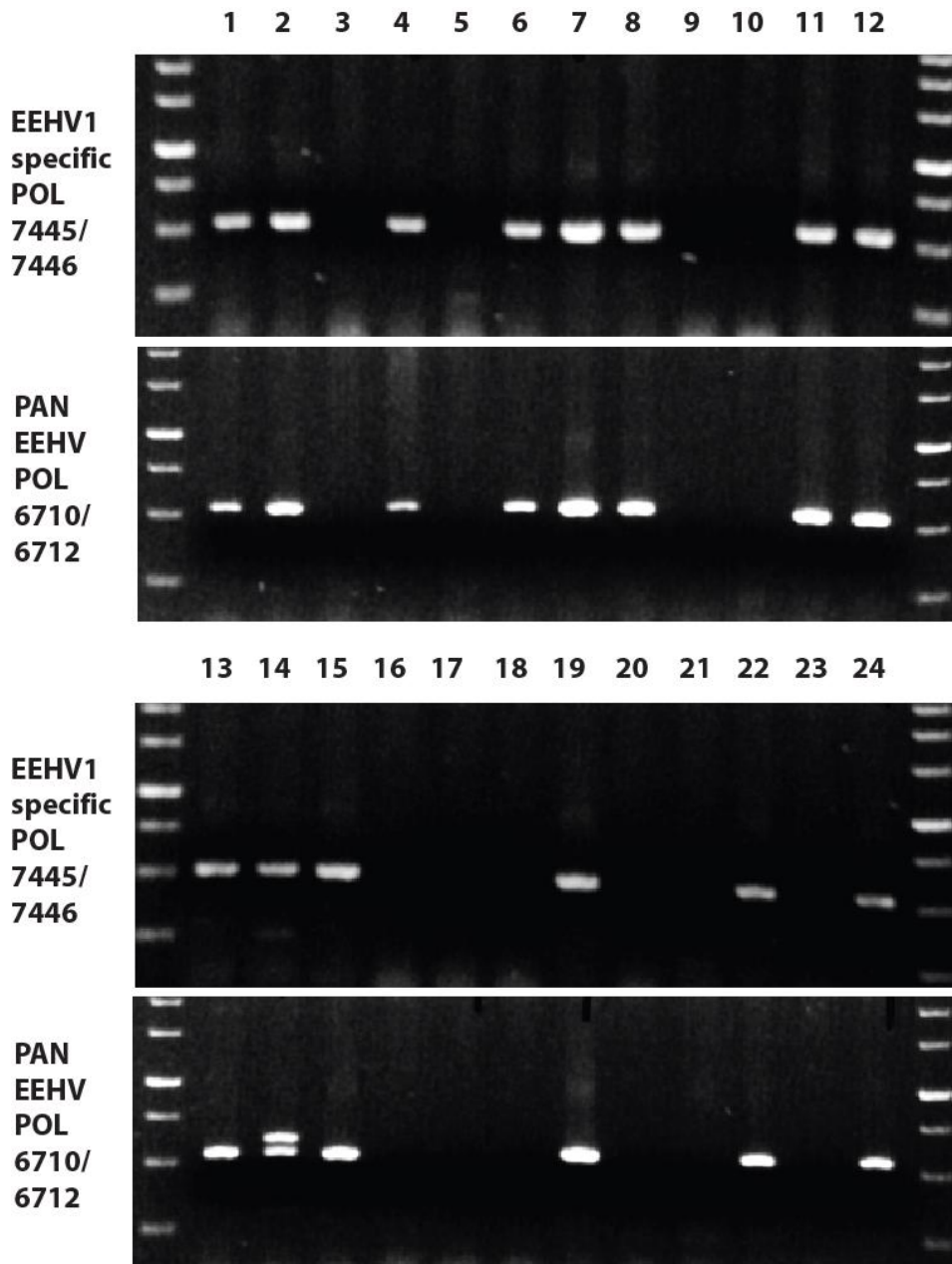
# Gross pathological lesions





# Inclusion Bodies





24x DNA samples from 16 *E.maximus*  
in Weyanad, Kerala, India.  
[20 x necropsy, 4 x blood samples]

First round positives for EEHV1:  
Lane Numbers:

Case #1: Niranjn = 7, 8, 13, 14, 24

Case #2: Aswathi = 1, 2, 12

Case #4 = 11, 19

Case #6 = 6

Case #7 = 7, 22

Case #14 = 15

[Cases #3 (23), #11 (5), #15 (9) were  
also positive after second round PCR]

8/9 hemorrhagic disease positive.  
0/3 control necropsy positive.  
1/4 live herdmate blood positive.



# IMPORTANT FINDINGS

**We have reported 31 cases EEHV1 A and one case of EEHV 1B with pathologic and molecular epidemiological evidences in Indian Subcontinent for the first time.**

**We have reported EEHV 1 A mortality free ranging elephants for the first time thus giving insight to the disease ecology and possible emergence of the virus.**

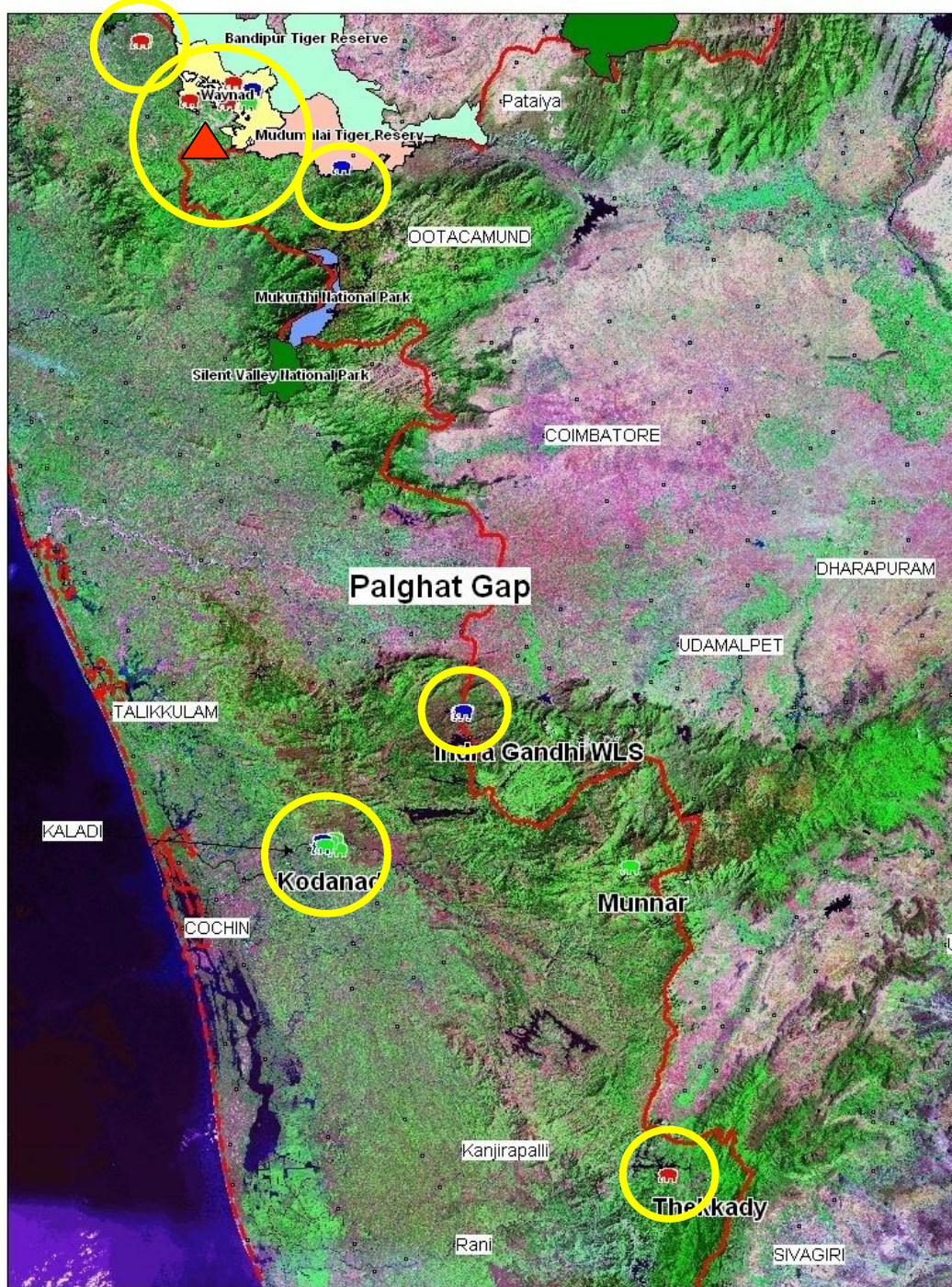
# Molecular epidemiology-strain sub typing

- In the beginning PCR amplification were performed in the core regions of 3,300 bp with 4 conserved region Pol, Ter, Hel and gM region and two hyper variable variable regions of gH-TK and vGPCR 1
- This was further extended to 6100 bp of genome by adding two terminal hyper variable region vOX2 vGPCR5 plus and extended version of U60(Ter ex3)

# **Molecular epidemiology-strain sub typing**

- 31 different EEHV 1A strain subtypes were identified showing high variability of the virus as shown in US and European strains
- However 13 novel strains were identified
- Spatial and temporal epidemiological correlations were not established
- Two cases that were infected and died in contact had 100% similarity sub strain indicating horizontal transmission.



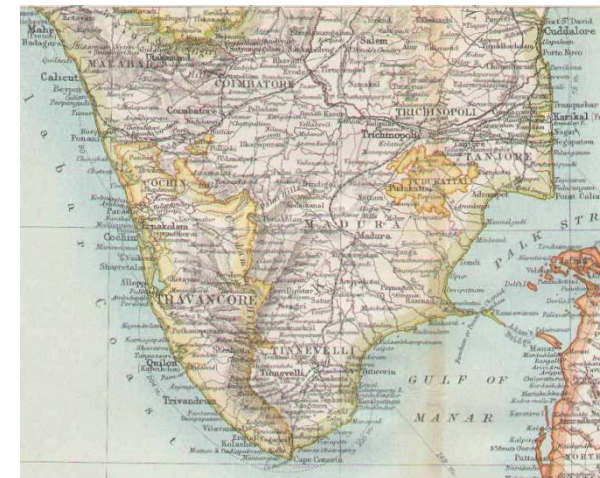


## South India Range :

**Kerala/ Tamil Nadu/ Karnataka :**

**18x Documented cases of Hemorrhagic Disease:**

- Red e-symbol= Wild born
- Blue e-symbol= Captive born





Transmission is horizontal  
EEHV in captive herd mates



# EEHV in Free-ranging herd mates

## Multiple strain circulating in a single herd





# Viral shedding in the captive Asian elephants in South India

## Detection of pathogenic elephant endotheliotropic herpesvirus in routine trunk washes from healthy adult Asian elephants (*Elephas maximus*) by use of a real-time quantitative polymerase chain reaction assay

Jeffrey J. Stanton, DVM; Jian-Chao Zong, PhD; Erin Latimer, BS; Jie Tan, BS; Alan Herron, DVM; Gary S. Hayward, PhD; Paul D. Ling, PhD

**Objective**—To investigate the pathogenesis and transmission of elephant endotheliotropic herpesvirus (EEHV) by analyzing various elephant fluid samples with a novel EEHV-specific real-time PCR assay.

**Animals**—5 apparently healthy captive Asian elephants (*Elephas maximus*) from the same herd.

**Procedures**—A real-time PCR assay was developed that specifically detects EEHV1. The assay was used to evaluate paired whole blood and trunk-wash samples obtained from the 5 elephants during a 15-week period. Deoxyribonucleic acid sequencing and viral gene subtyping analysis were performed on trunk-wash DNA preparations that had positive results for EEHV1. Viral gene subtypes were compared with those associated with past fatal cases of herpesvirus-associated disease within the herd.

**Results**—The PCR assay detected viral DNA to a level of 1,200 copies/mL of whole blood. It was used to detect EEHV1 in trunk secretions of 3 of the 5 elephants surveyed during the 15-week period. Viral gene subtyping analysis identified 2 distinct elephant herpesviruses, 1 of which was identical to the virus associated with a previous fatal case of herpesvirus-associated disease within the herd.

**Conclusions and Clinical Relevance**—EEHV1 was shed in the trunk secretions of healthy Asian elephants. Trunk secretions may provide a mode of transmission for this virus. Results of this study may be useful for the diagnosis, treatment, and management of EEHV1-associated diseases and the overall management of captive elephant populations. (*J Am Vet Res* 2010;71:925–933)

Elephant endotheliotropic herpesviruses can cause acute hemorrhagic disease in endangered Asian and African (*Loxodonta africana*) elephants, resulting in considerable illness, reproductive loss, and death in captive elephant populations.<sup>1,2</sup> This herpesvirus-associated disease primarily affects juvenile Asian elephants and results in rapid-onset endotheliotropic disease with a mortality rate of 85% in elephants that have positive results for the disease as indicated by semiquantitative conventional PCR-assay blood tests.<sup>3</sup> After the origi-

### ABBREVIATIONS

C	Cycle threshold
EEHV	Elephant endotheliotropic herpesvirus
EGHV	Elephant gammaherpesvirus
gM	Glycoprotein M
IFN- $\gamma$	Interferon $\gamma$
MDBP	Major DNA-binding protein
NAP	North American proboscivirus
POL	DNA polymerase
qPCR	Quantitative PCR
VGC	Viral genome copy
vGPCR	Viral G-protein coupled receptor

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From the Department of Molecular Virology and Microbiology (Stanton, Tan, Ling) and the Center for Comparative Medicine and Department of Pathology and Immunology (Herron), Baylor College of Medicine, Houston, TX 77030; Viral Oncology Program, School of Medicine, Johns Hopkins University, Baltimore, MD 21201 (Zong, Hayward); and Elephant Herpesvirus Laboratory, Smithsonian National Zoological Park, 3001 Connecticut Ave NW, Washington, DC 20013 (Latimer).

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925

- No of individuals tested  
48 elephants from 3 camps
- 2 elephants shed EEHV 1A virus
- One elephant sheds EEHV5 virus.

# Viral shedding in the Free ranging wild Asian elephants in South India



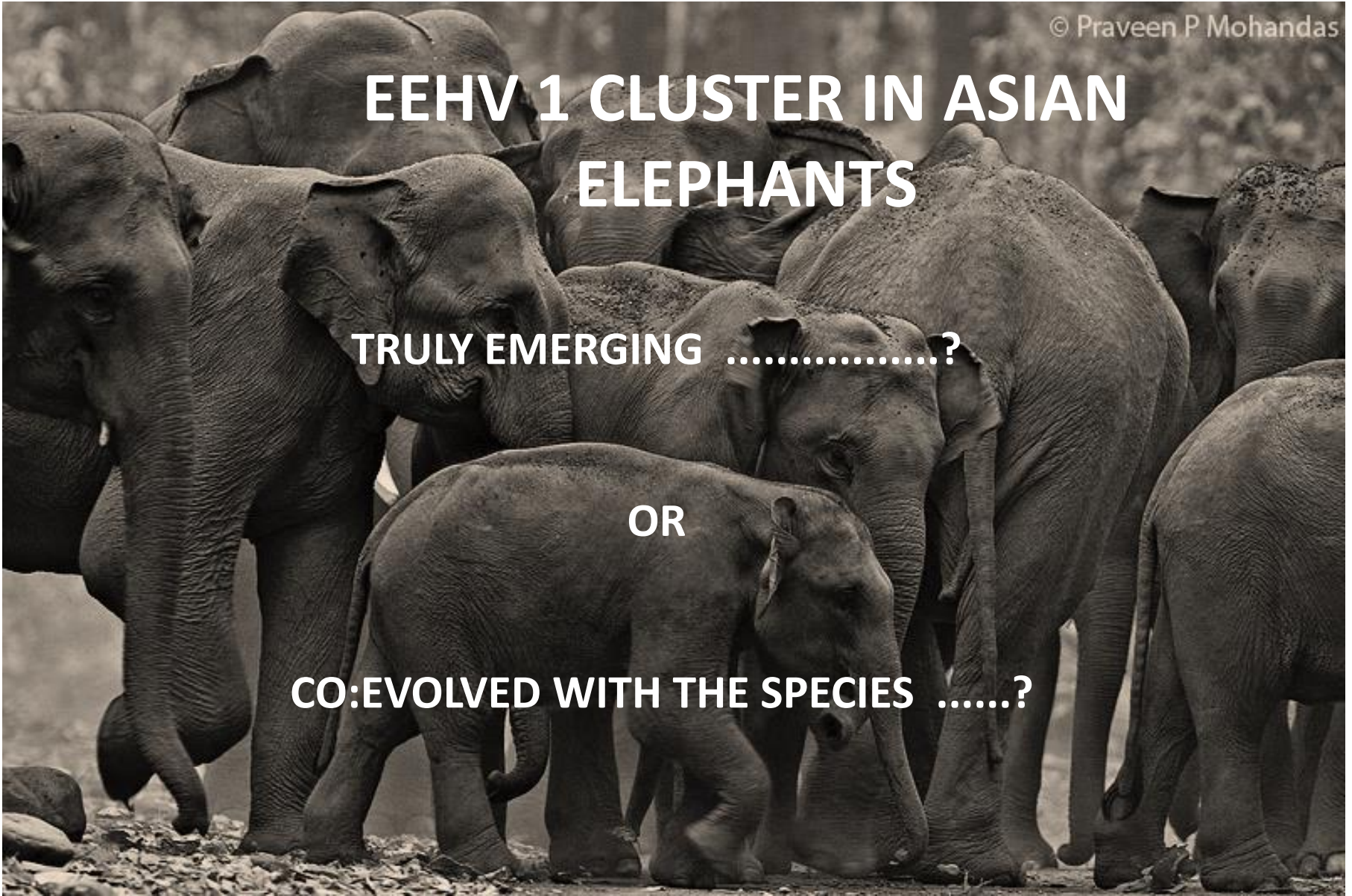
- No of individuals tested:  
22 elephants from Wayanad  
wildlife sanctuary
- 2 elephants shed EEHV 1A  
virus and 4 has viremia

# EEHV 1 CLUSTER IN ASIAN ELEPHANTS

TRULY EMERGING .....?

OR

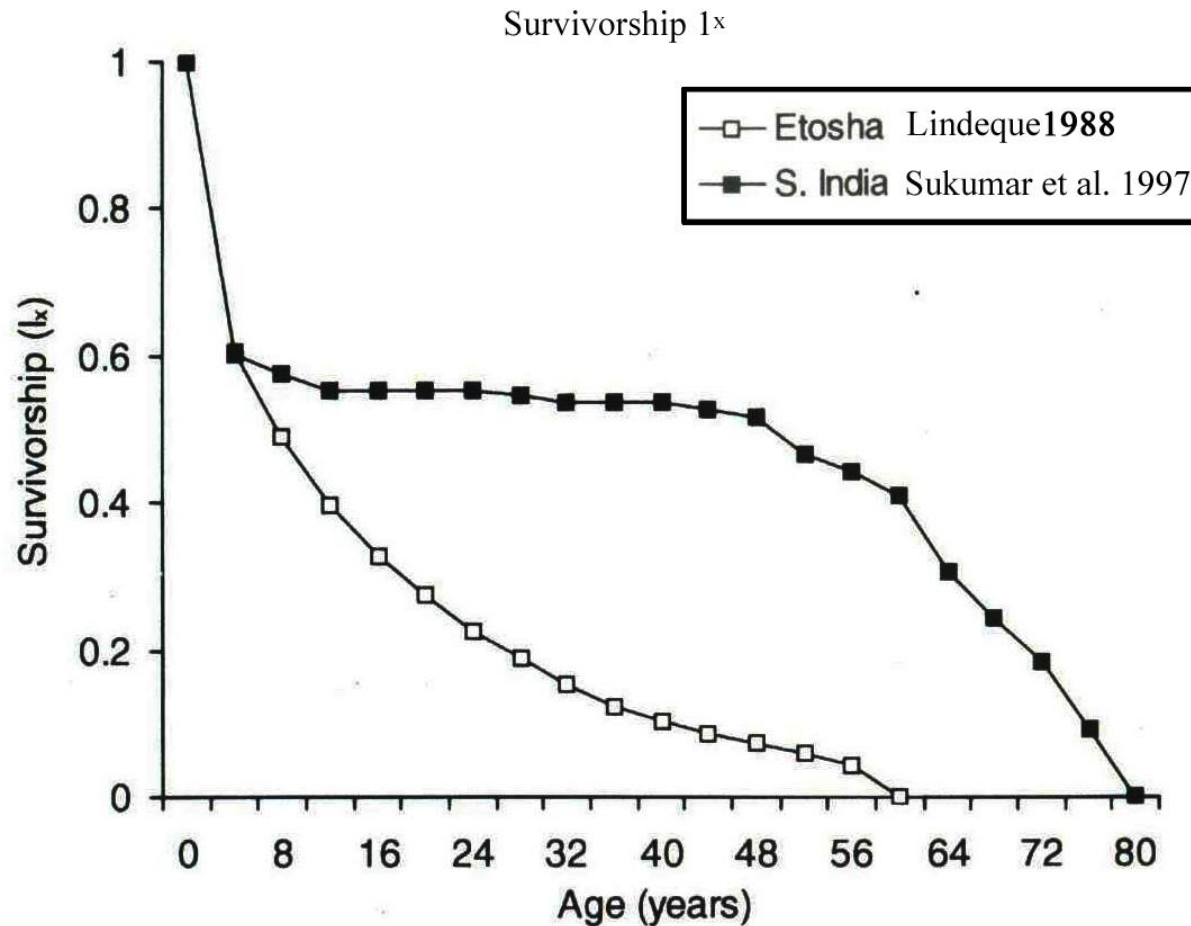
CO:EVOLVED WITH THE SPECIES .....?





# IMPACT ON THE SURVIVAL OF THE SPECIES

## Survivorship 1x curve for African and Asian elephants



# **Projects undertaking for characterization of host genotype and development of markers for population risk assessment**

1. Whole genome sequencing of 16 strains to understand viral evolution
2. Identification of MHC genotype mapping
3. Host genotyping in EEHV mortality and possible development of markers (Genetic association studies)



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THANK YOU

