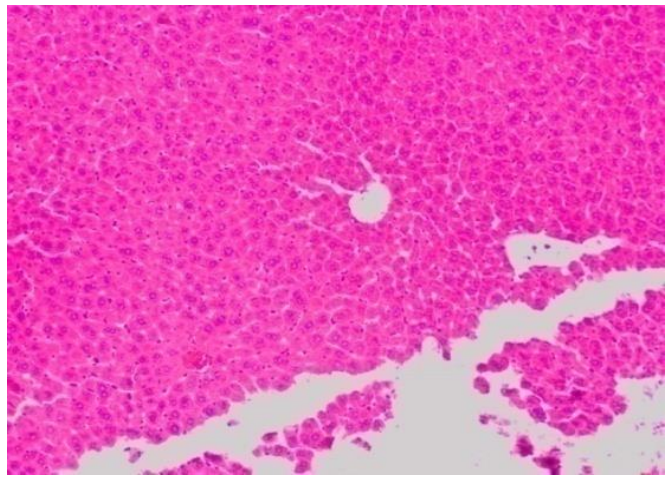
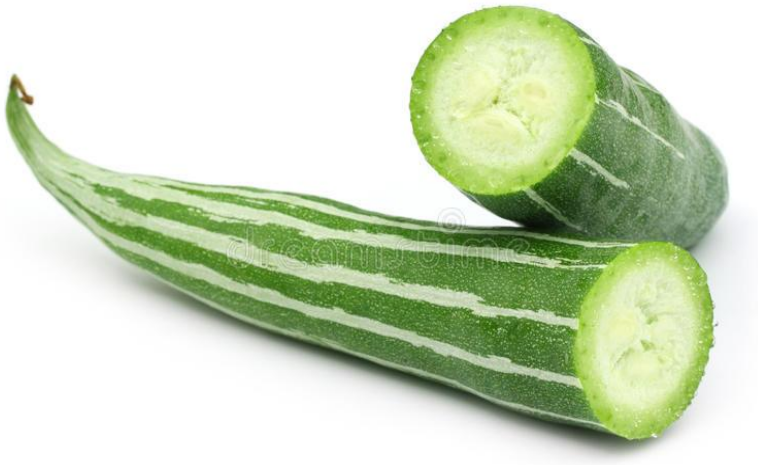
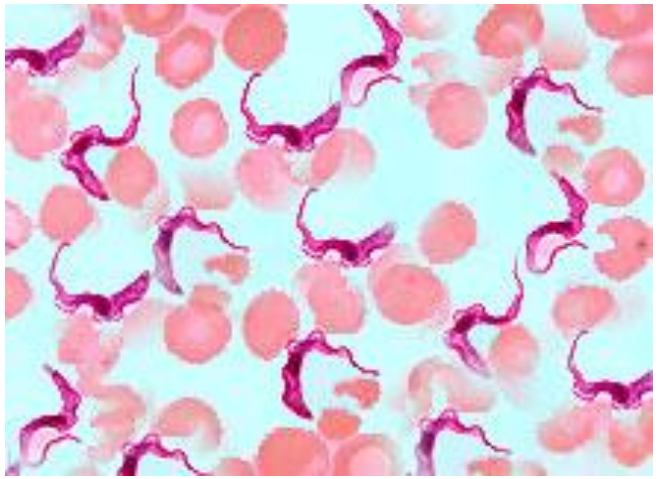
OP-01
MOHD SHUKRI BABA
(MALAYSIA)

IN-VIVO ANTIPARASITIC ACTIVITY AND TOXICITY EVALUATION OF *Trichosanthes cucumerina* AGAINST THE GROWTH AND SURVIVAL OF ZOONOTIC HAEMOFLAGELLATE, *Trypanosoma evansi* IN MICE







Asst. Prof. Dr. Mohd Shukri Baba

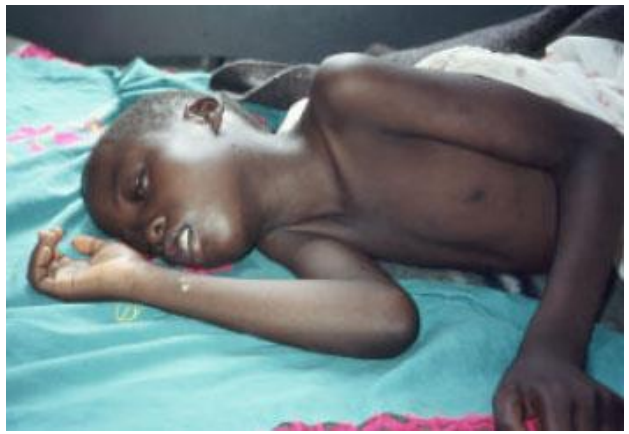
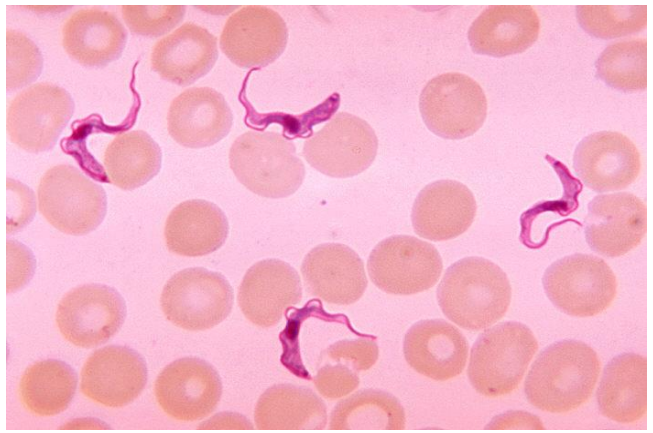
International Islamic University Malaysia

INTRODUCTION

Trypanosoma evansi

- First discovered by Sir Griffith Evans in Punjab India (1880)
- Haemoflagellated protozoa in both human and animals → zoonotic vector-borne disease
- Caused atypical human trypanosomiasis (AHT) in human and Surra disease in mammals (mostly livestock)
- Wide variety of vectors → worldwide distributed
- Drug resistant in some regions → suramin, pentamidine, berenil



Vectors of *Trypanosoma evansi*

Hirudo medicinalis

Tabanus striatus

Desmondus rotundus

Triatoma infestants

Glossina morsitans

Anthomyia pluvialis

Trichosanthes cucumerina : The Testimonial

Antibacterial activities (Reddy et al. 2010)
- *B. cereus, S. aureus, E. coli, S. feacalis, P. aeruginosa, K. pneumoniae, S. typhi.*

Larvicidal efficacy (Rahuman & Vankatesan, 2008)
- L4 of *Ae. aegypti*
- L4 of *Cu. quinquefasciatus*

Anticancer activities (Kongtun et al. 2009)
- Human breast cancer cell lines (SKBR3, MCF7, T47D & MDA-MB435),
- Human lung cancer cell lines (A549 and SK-LU1)
- Human colon cancer cell line (Caco-2)

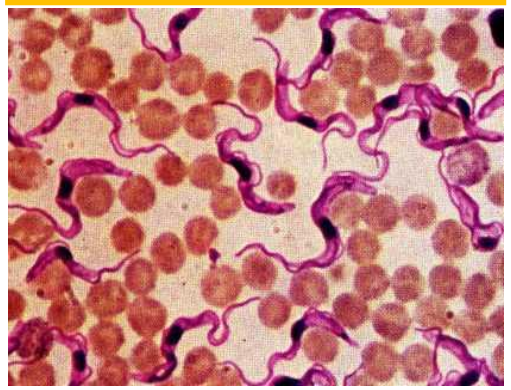
Antidiabetic activities (Kirana & Srinivasan, 2008)
- 72% improved at 90 mg/kg bw (i.p.) on NIDDM rats

Hepatoprotective activities (Arawwawala et al. 2010)
- Methanolic extract towards inhibition of lesion length at 750 mg/kg

MATERIALS & METHODS

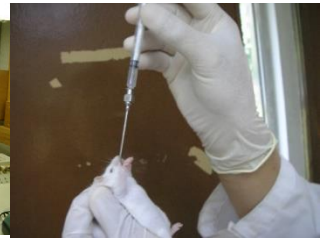
Flow Chart

T. evansi stock



T. evansi administered *i.p.* (5 × 10^3 *T. evansi* / mice)

Orally administered of 0.2 mL 100 mg/kg bw of freeze-dried *T. cucumerina* aqueous exract



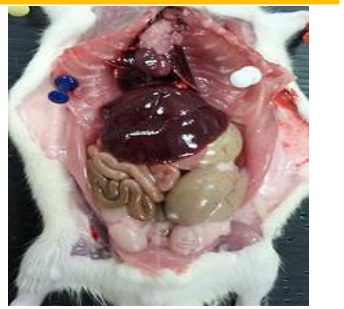
Giemsa blood slide for inhibition rate evaluation

Blood slide for electron microscopic observation

Physical observation of symptoms and mice survival

Blood biochemistry and renal function tests

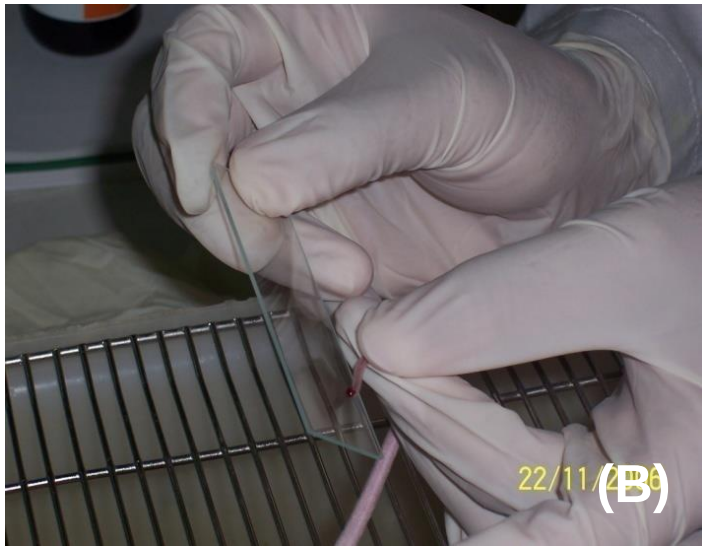
Vital organ histology for toxicity assessment

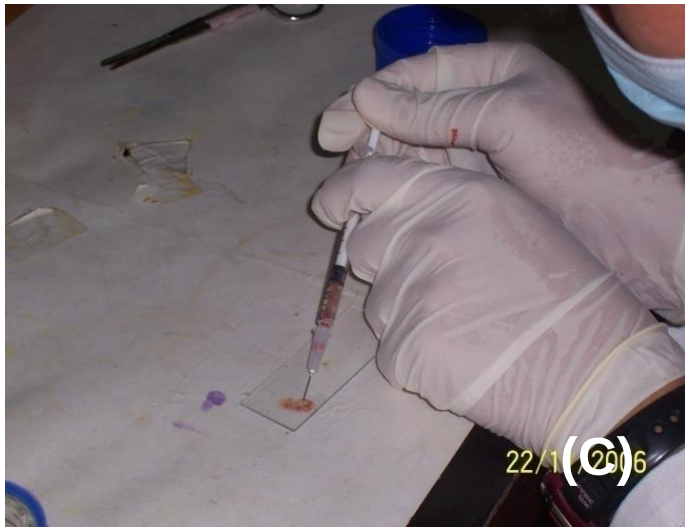


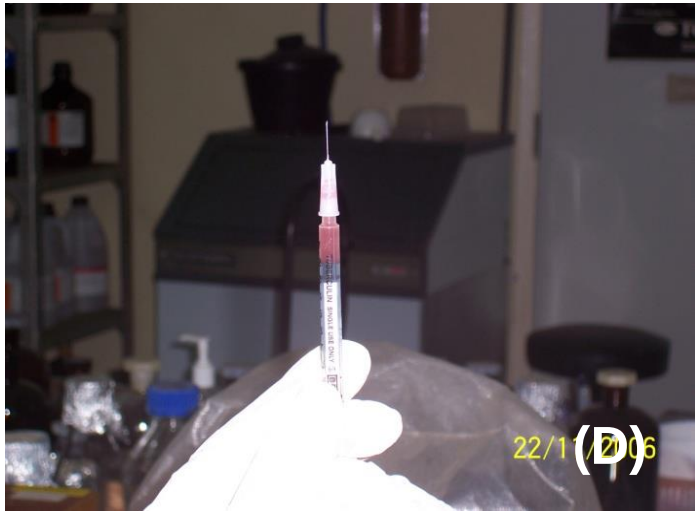
Experimental Design

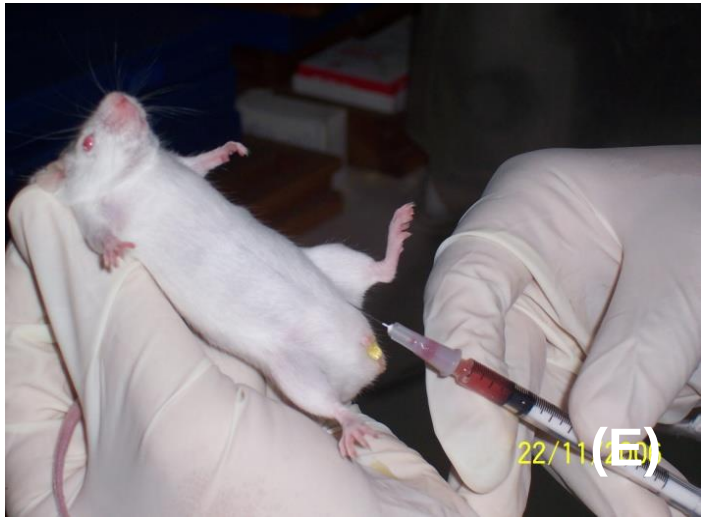
GROUP	REGIME	DESCRIPTION	*T. cucumerina* DOSAGE
TREATMENT	PREVENTIVE	14 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
		7 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
		3 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
	CURATIVE	3 days post-infection	0.2 mL 100 mg/kg bw aqueous-extract
		5 days post-infection	0.2 mL 100 mg/kg bw aqueous-extract
		7 days post-infection	0.2 mL 100 mg/kg bw aqueous-extract
GROUP	**REGIME**	**DESCRIPTION**	**CONTROL DOSAGE**
CONTROL	POSITIVE	Berenil (Sigma-Aldrich KL)	0.01 mL 3.5 mg/kg bw
	NEGATIVE	0.9 % Normal Saline	0.1 mL 0.9 normal saline
	LETHAL INFECTION	Infection without treatment	5×10^3 *T. evansi* / mice (i.p.)

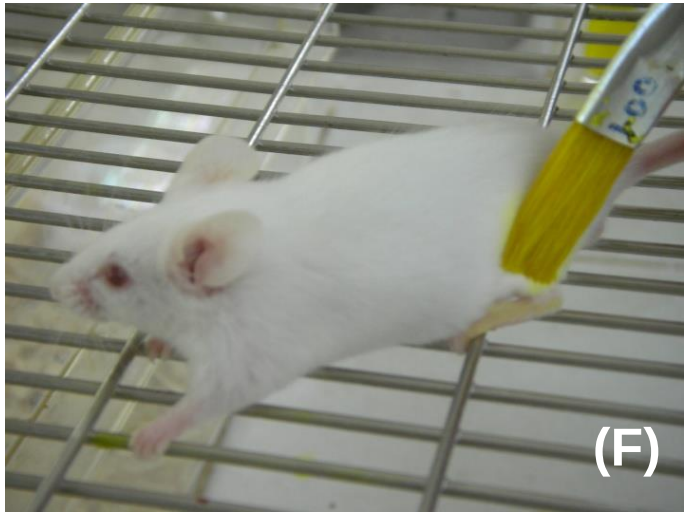
Parasite Administration And Animal Tagging



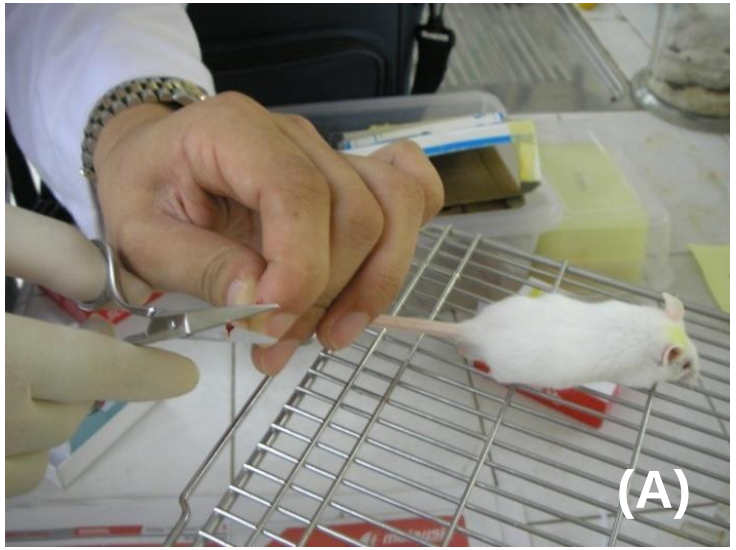


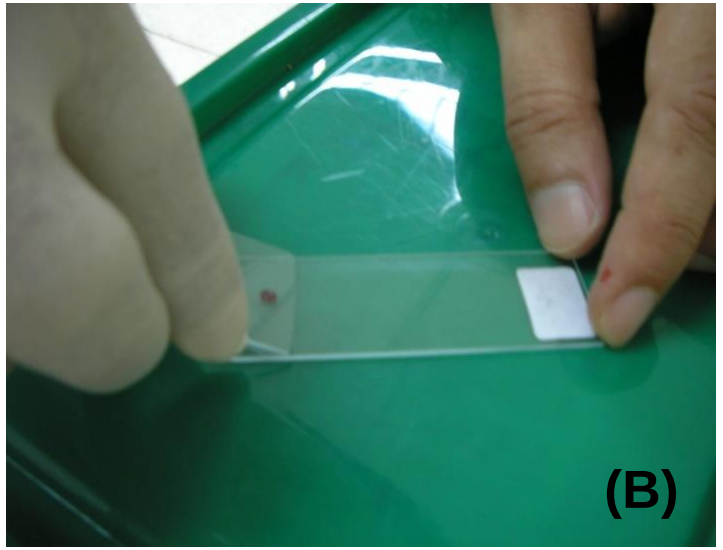


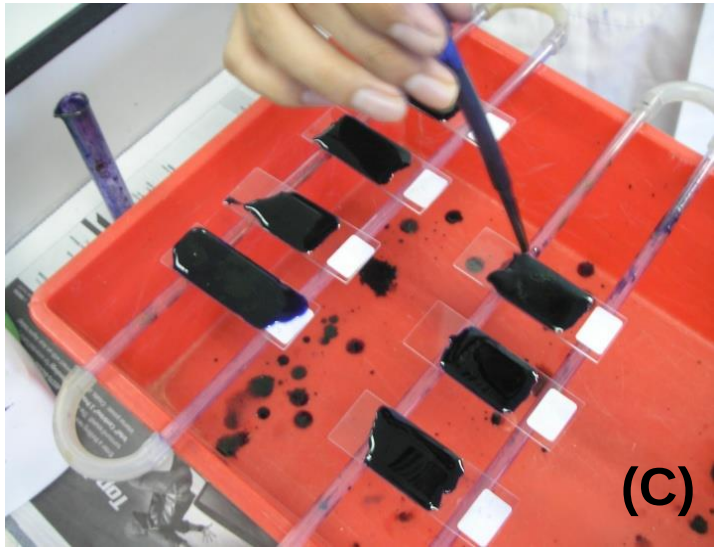


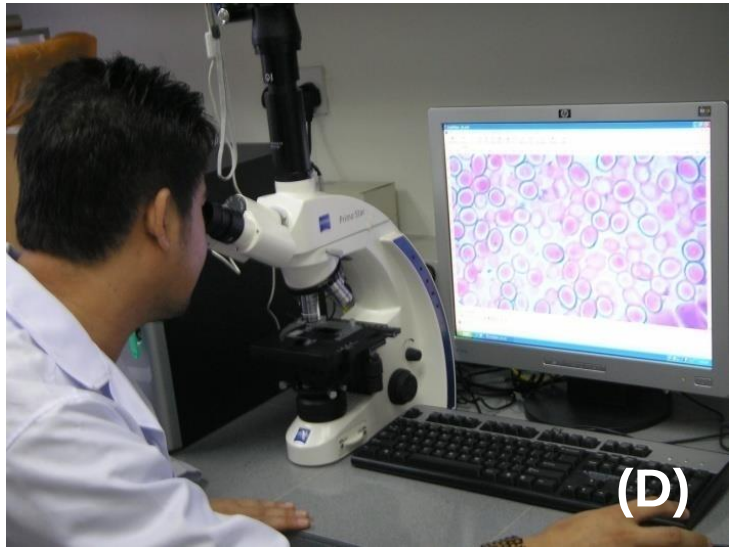


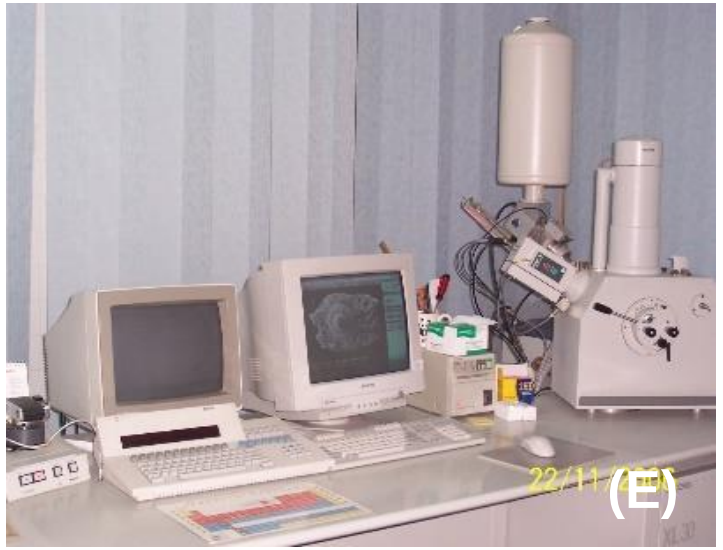
Giemsa Staining And Microscopic Observation

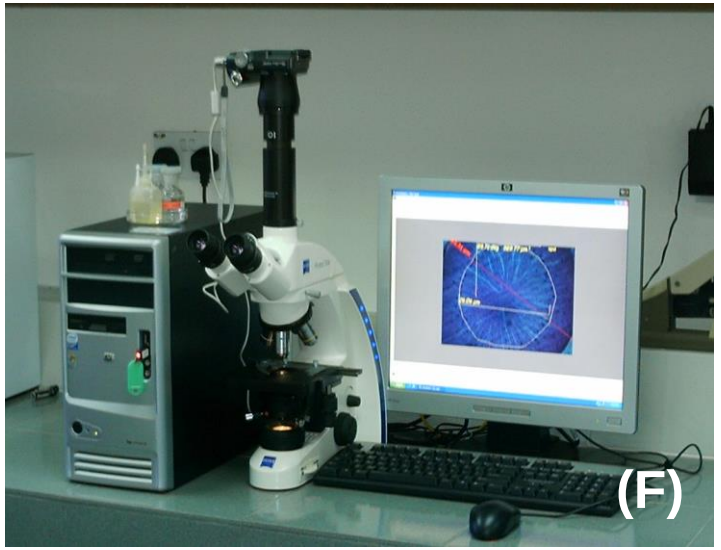




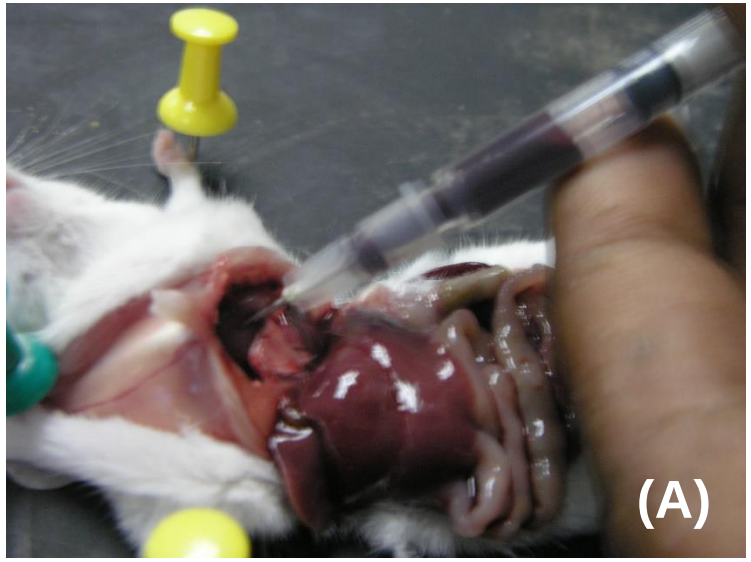


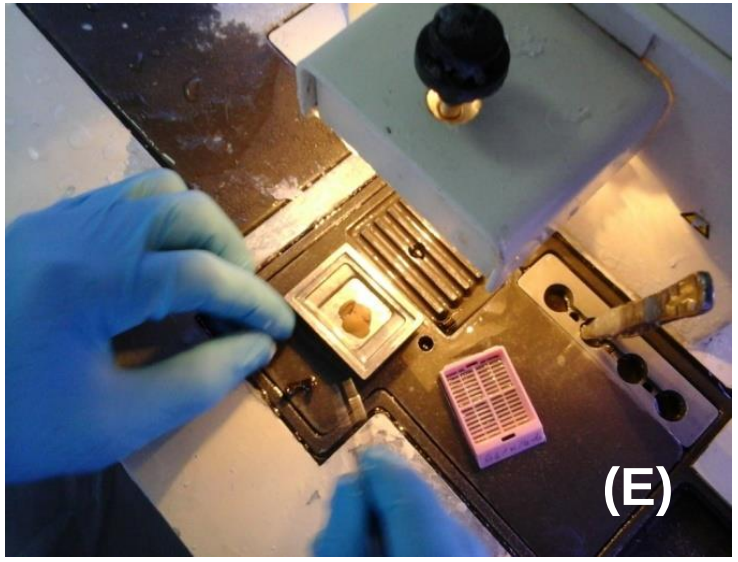






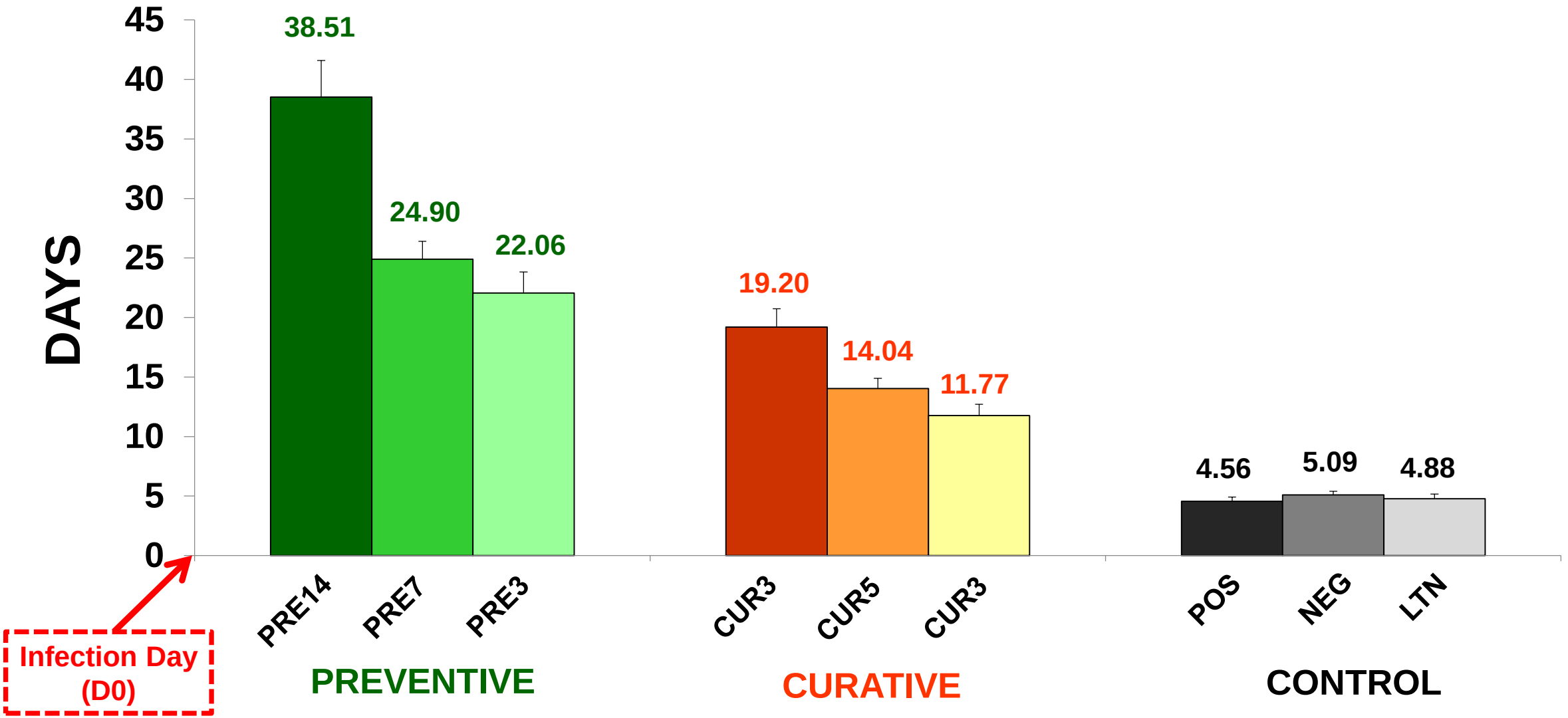
Biochemical Test And Histology Of Liver & Kidney



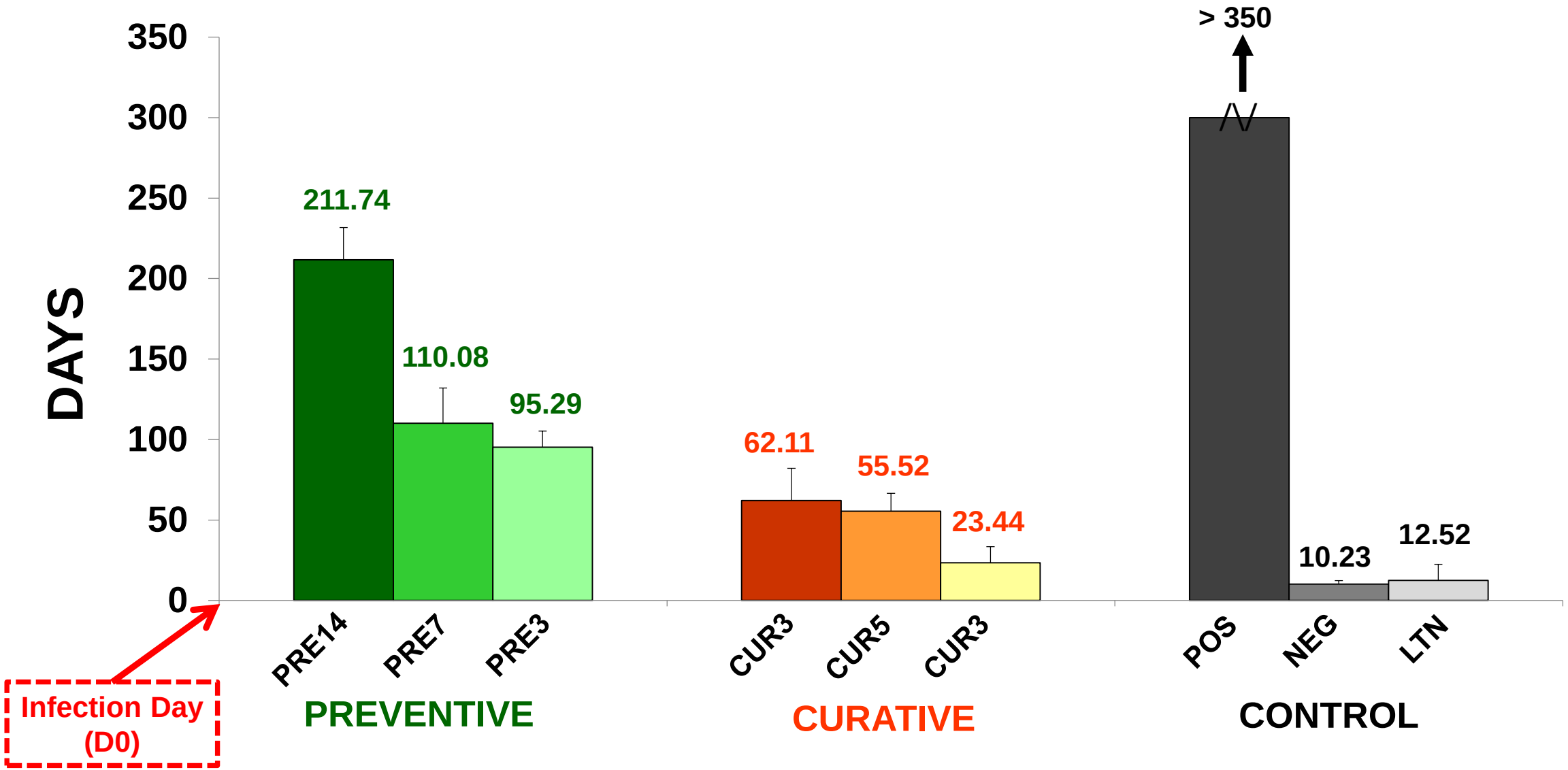


RESULTS & DISCUSSIONS

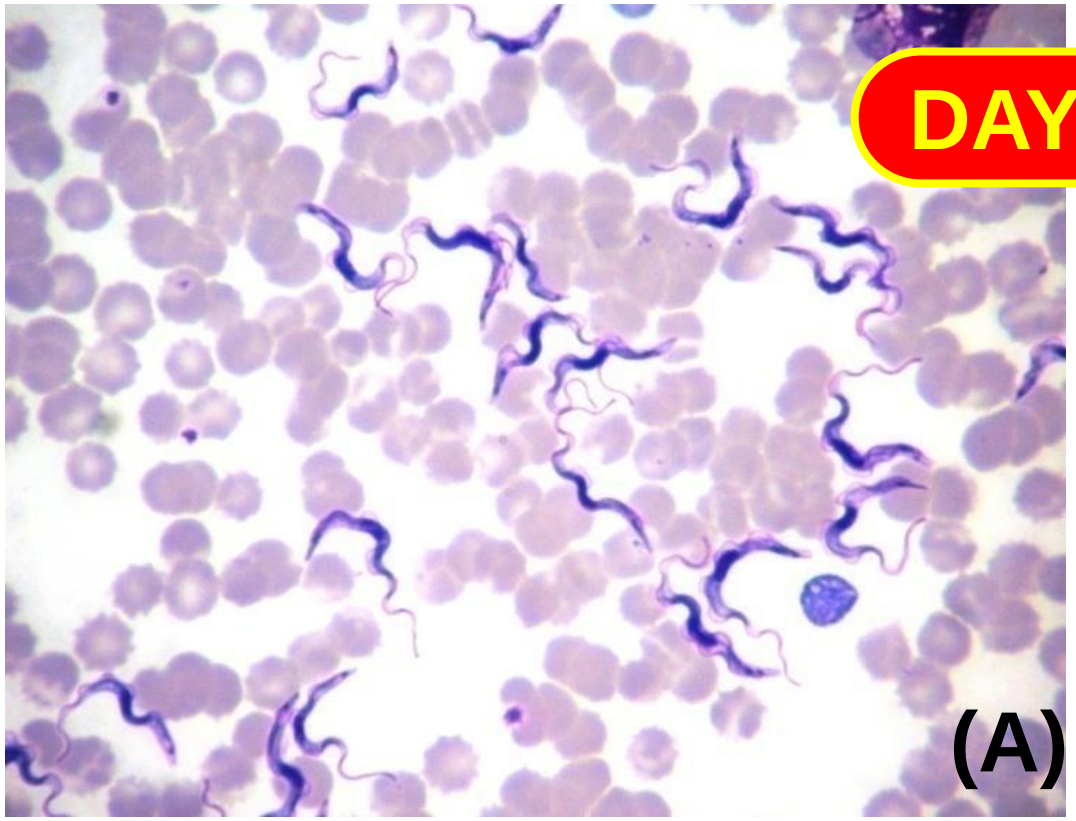
Parasite Pre-Patent Period (Day)

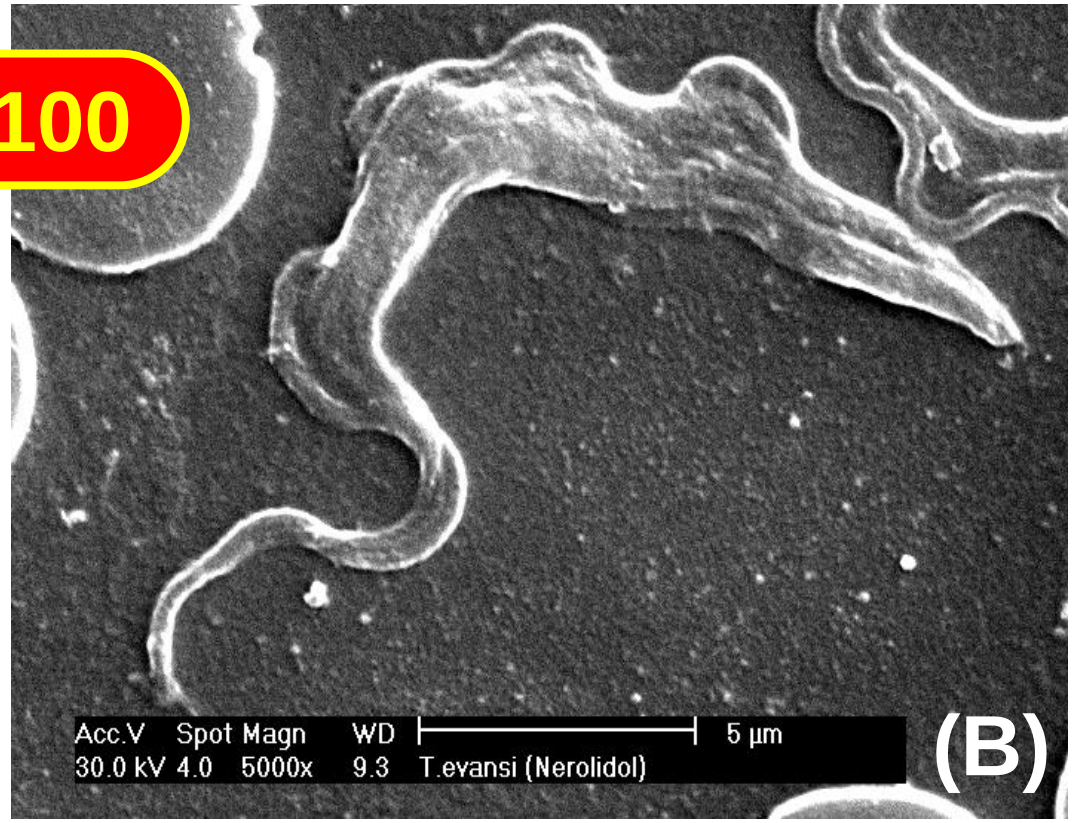


Mice Survival Time (Day)



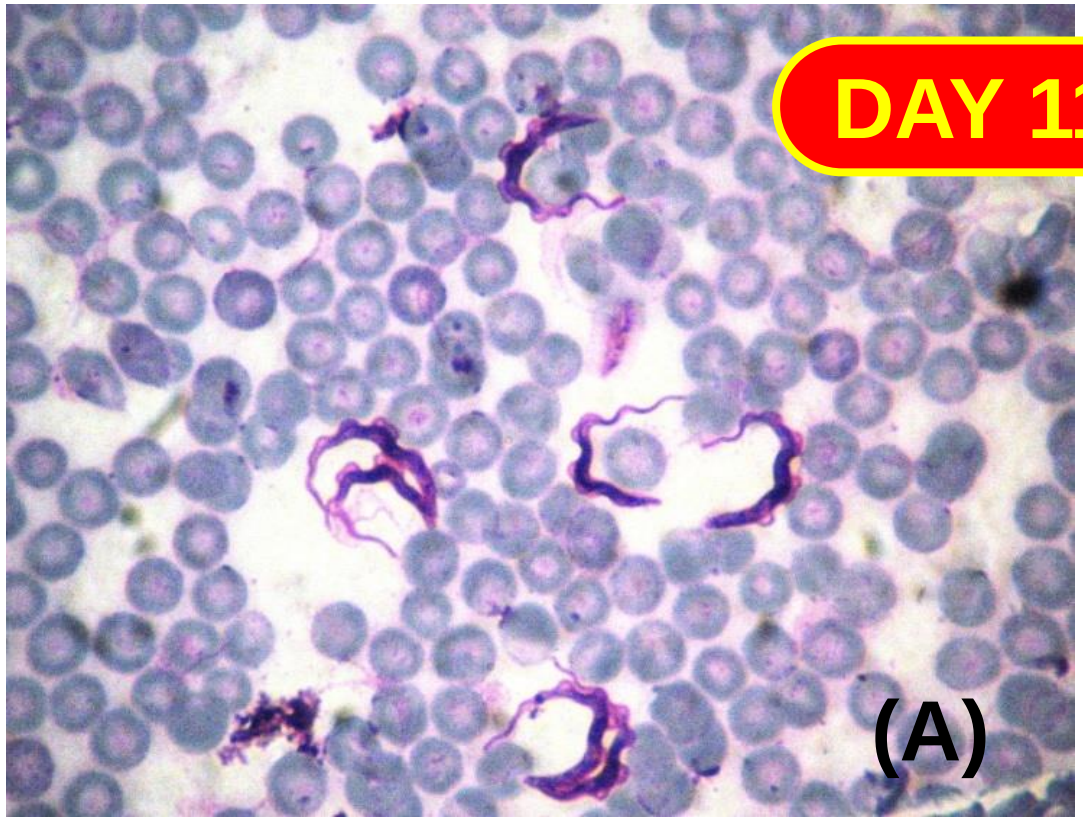
Parasite Survival In PRE14 Mice Group : 100th Day

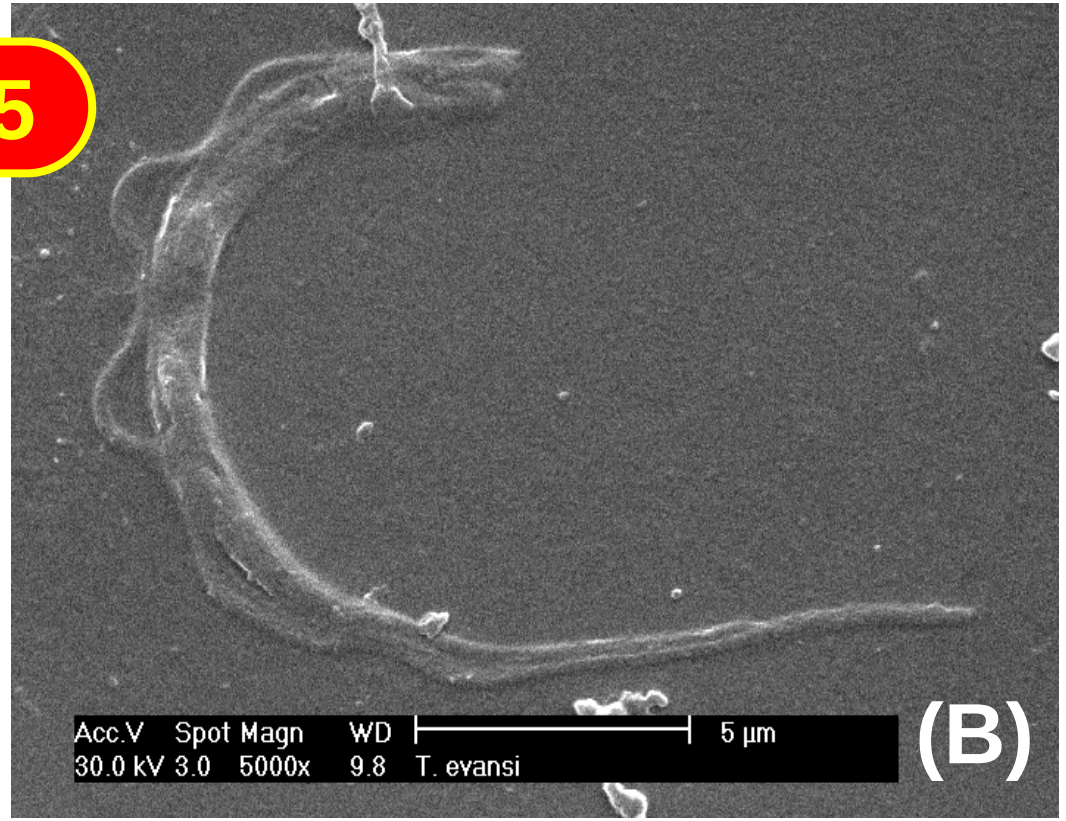




Giemsa thin blood smear of the mice from PRE14 mice group taken on day 100 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

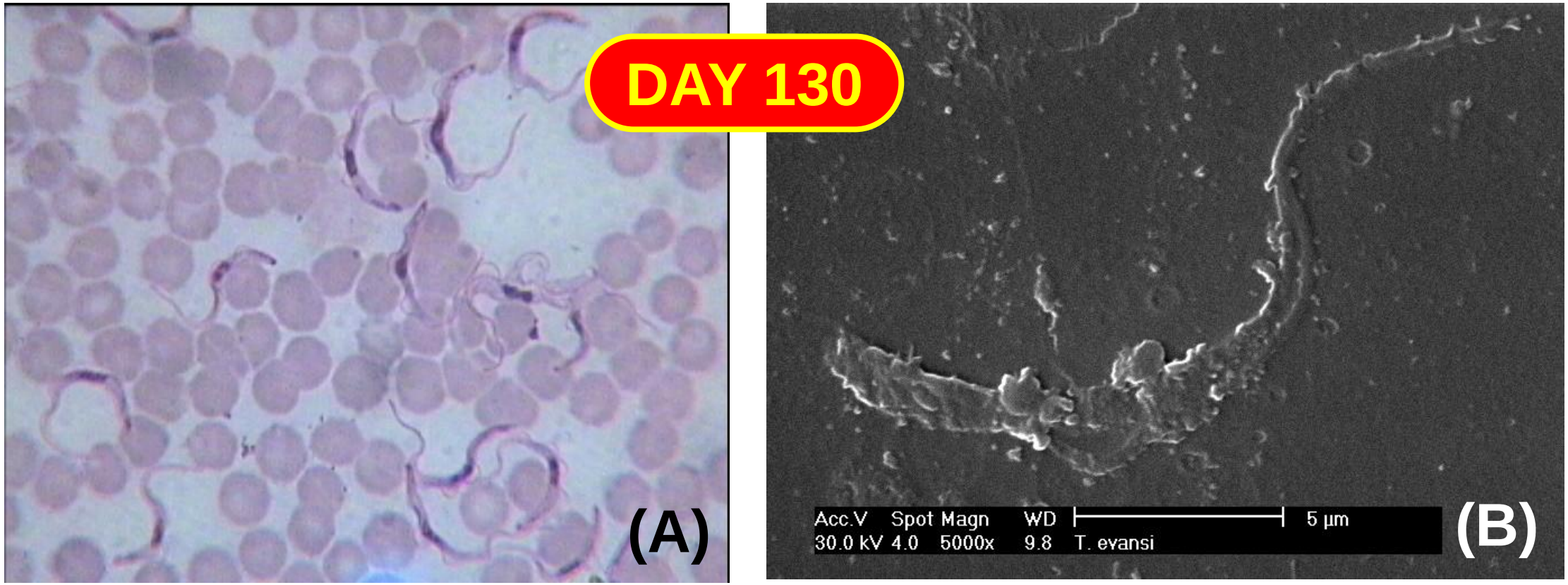
Parasite Survival In PRE14 Mice Group : 115th Day





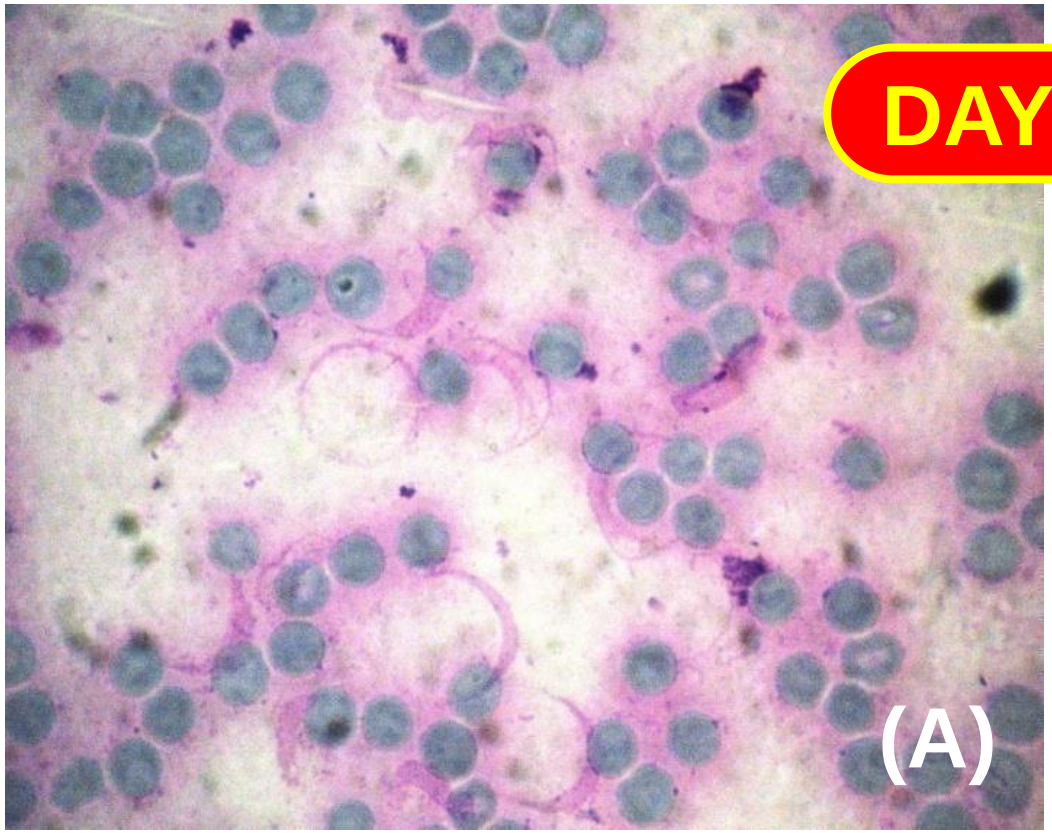
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 115 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

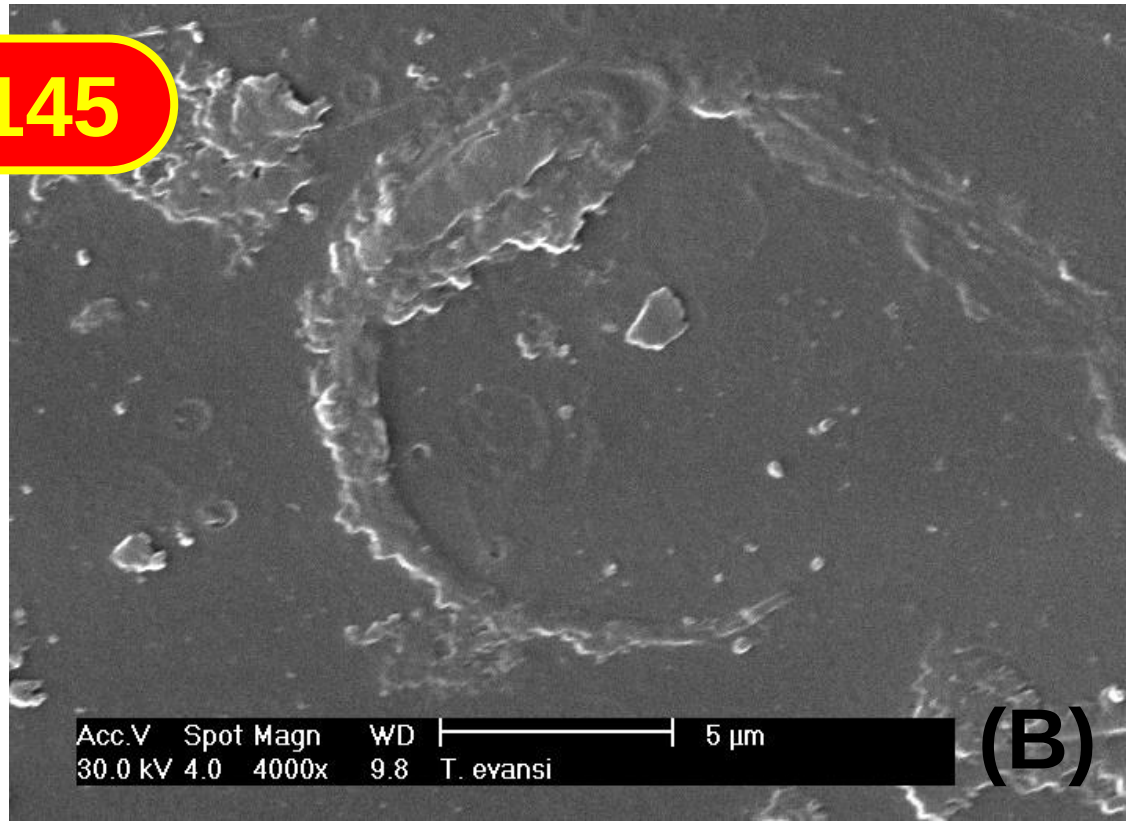
Parasite Survival In PRE14 Mice Group : 130th Day



Giemsa thin blood smear of the mice from PRE14 mice group taken on day 130 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

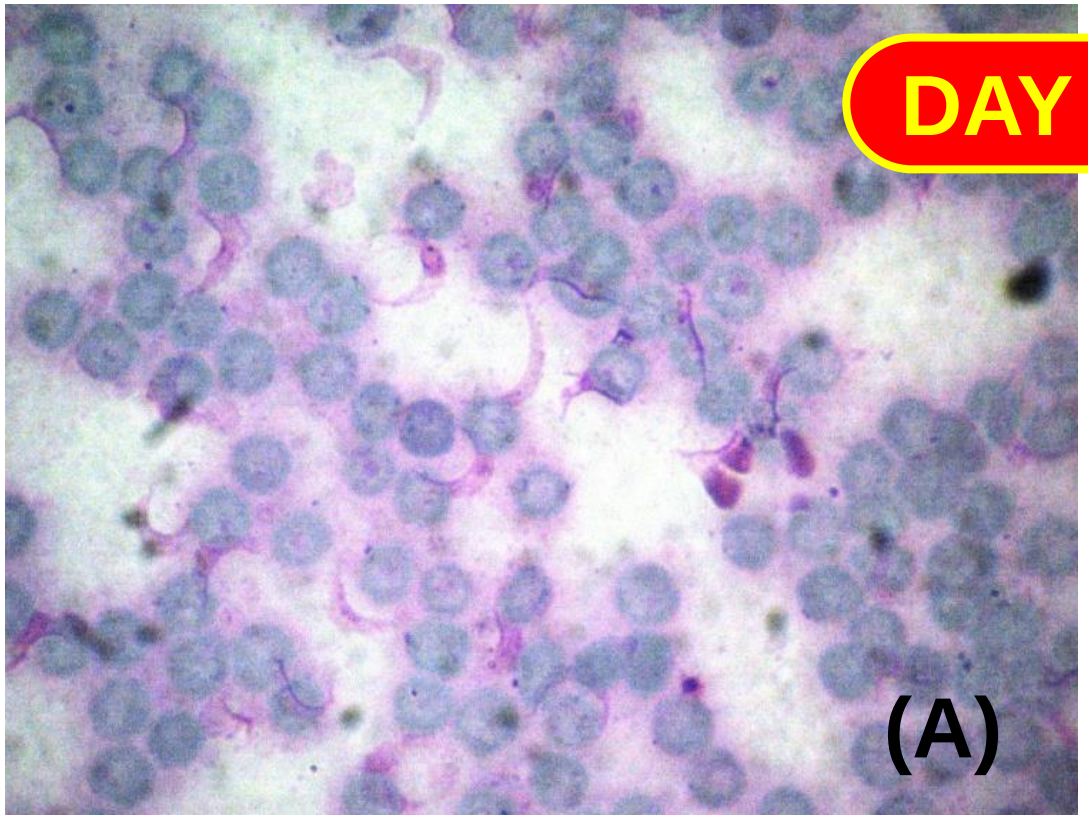
Parasite Survival In PRE14 Mice Group : 145th Day

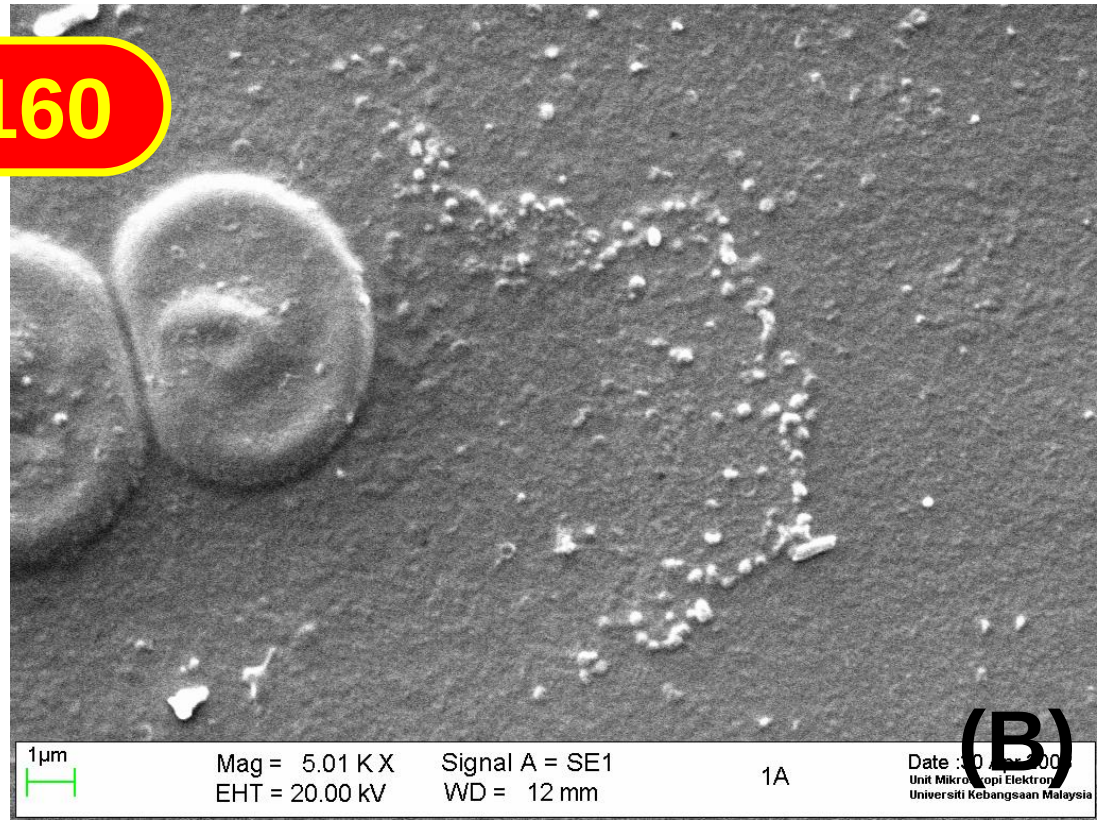




Giemsa thin blood smear of the mice from PRE14 mice group taken on day 145 post-infection as observed under x100 magnification of light microscope (A) and x4000 magnification of SEM (Phillips XL30, UK) (B)

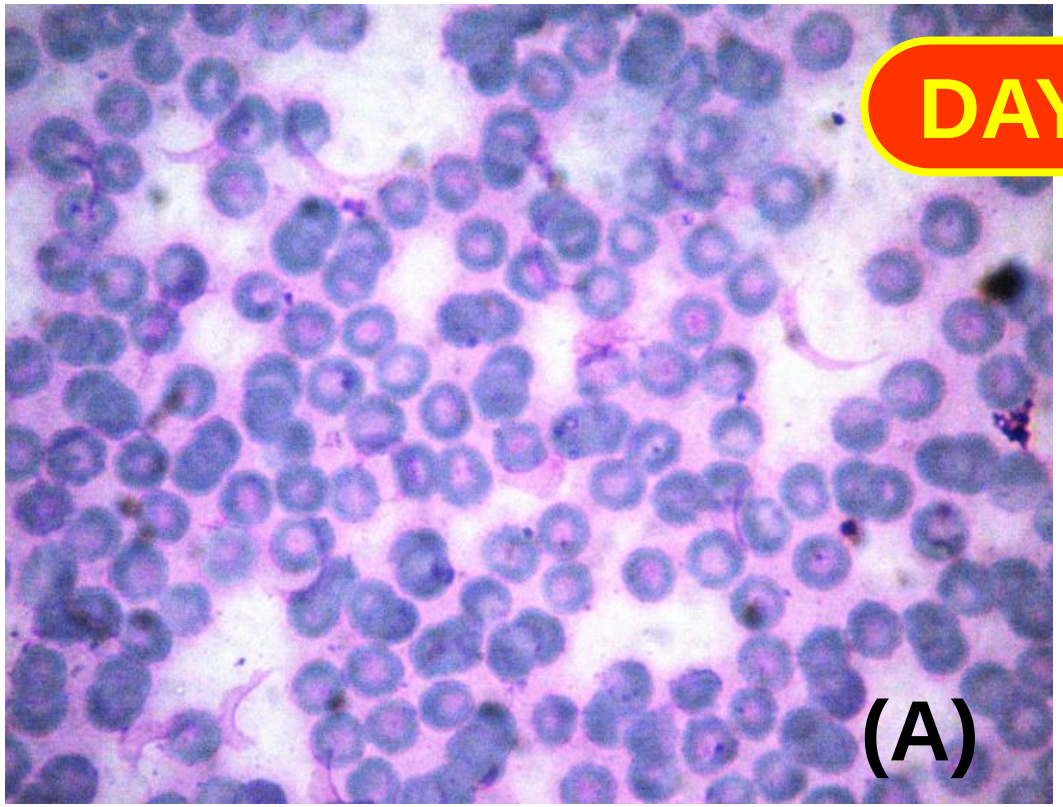
Parasite Survival In PRE14 Mice Group : 160th Day

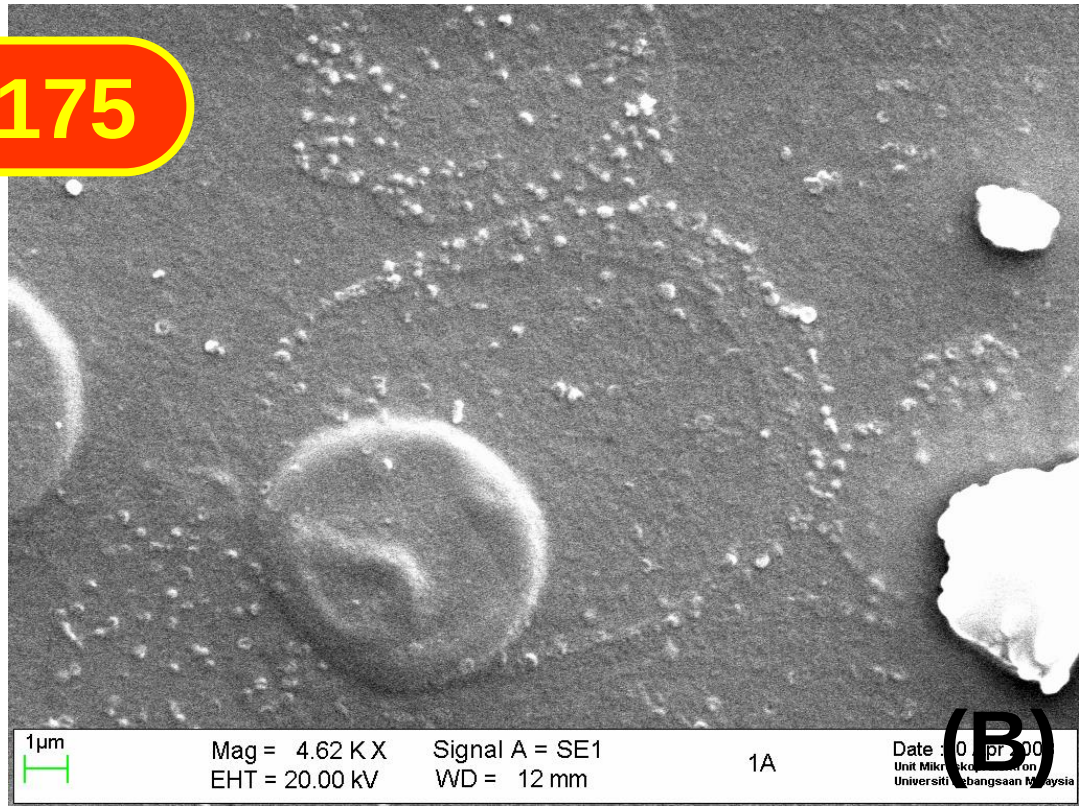




Giemsa thin blood smear of the mice from PRE14 mice group taken on day 160 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Leo 1450VP, Japan) (B)

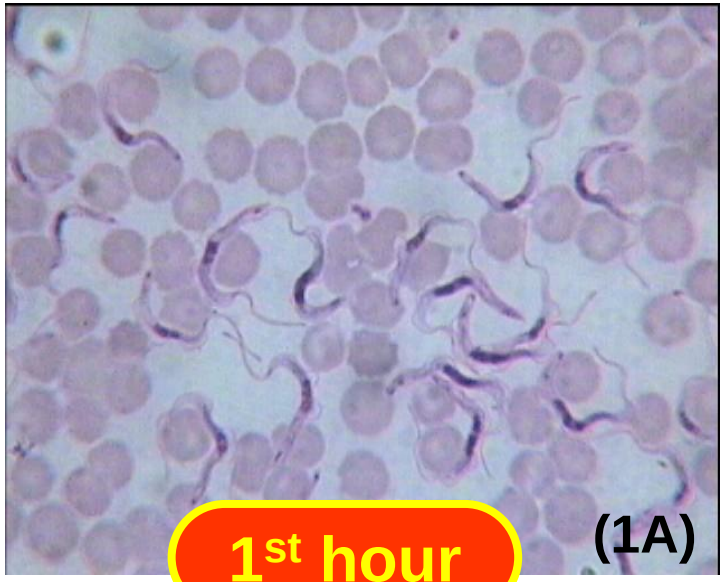
Parasite Survival In PRE14 Mice Group : 175th Day





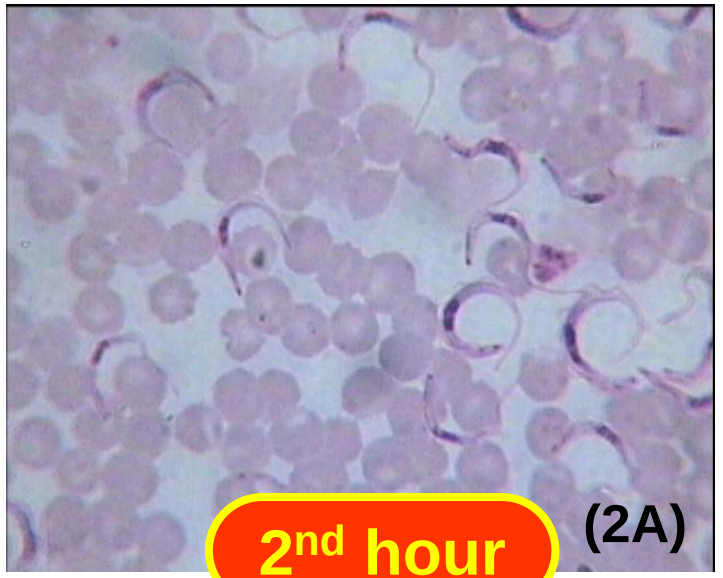
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 160 post-infection as observed under x100 magnification of light microscope (A) and x4600 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Growth in Berenil-Treated Group (POS)



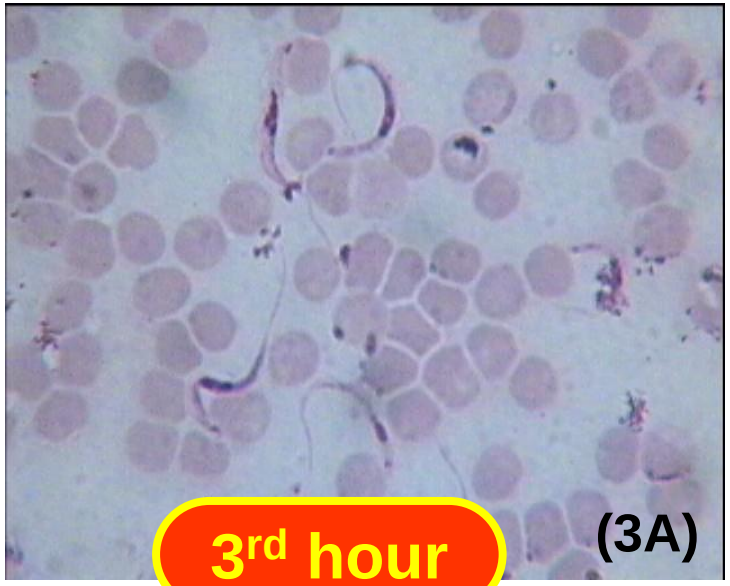
1st hour

(1A)



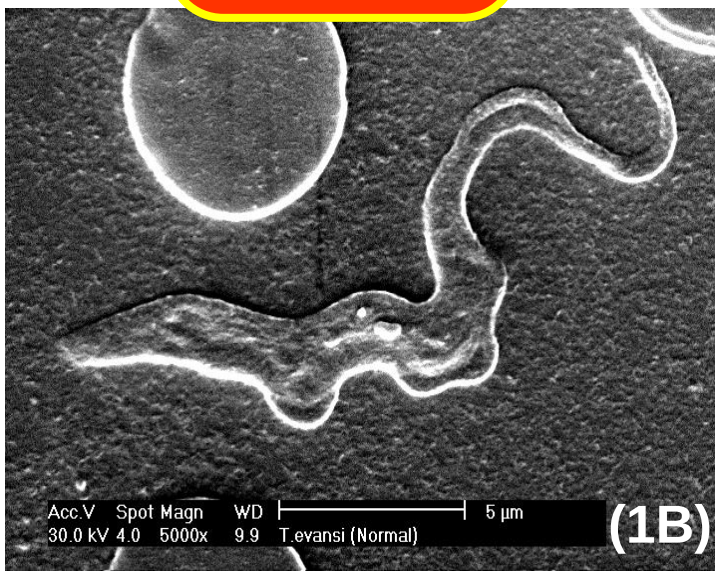
2nd hour

(2A)

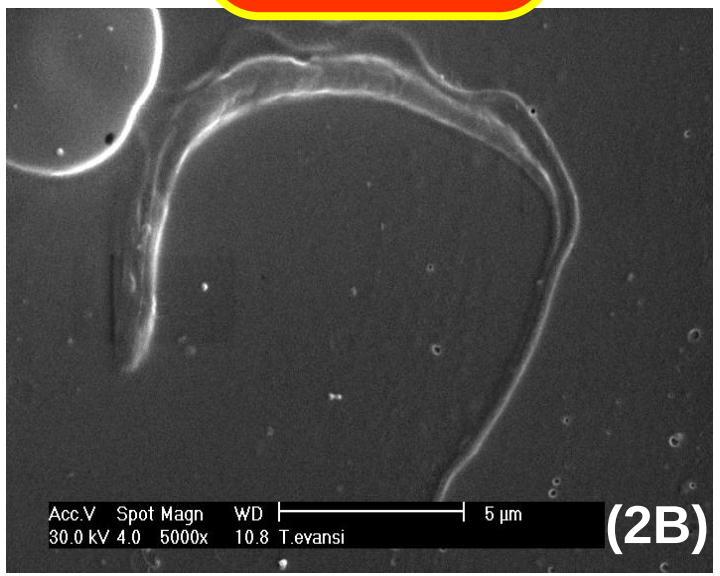


3rd hour

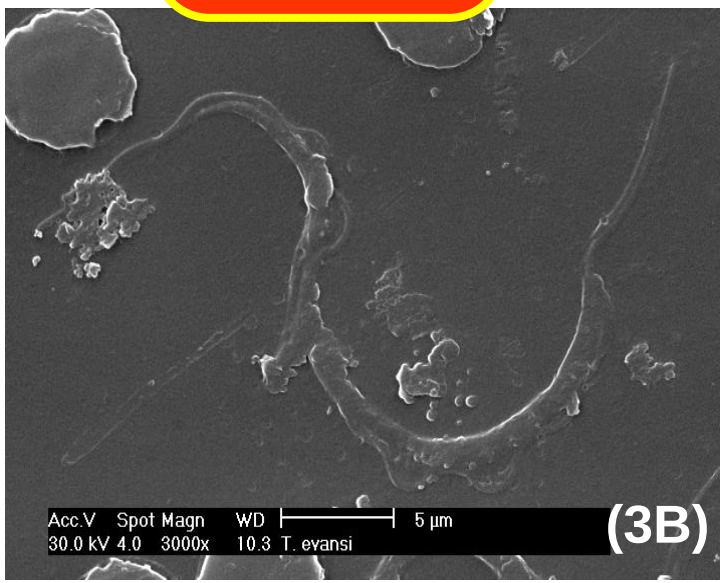
(3A)



(1B)

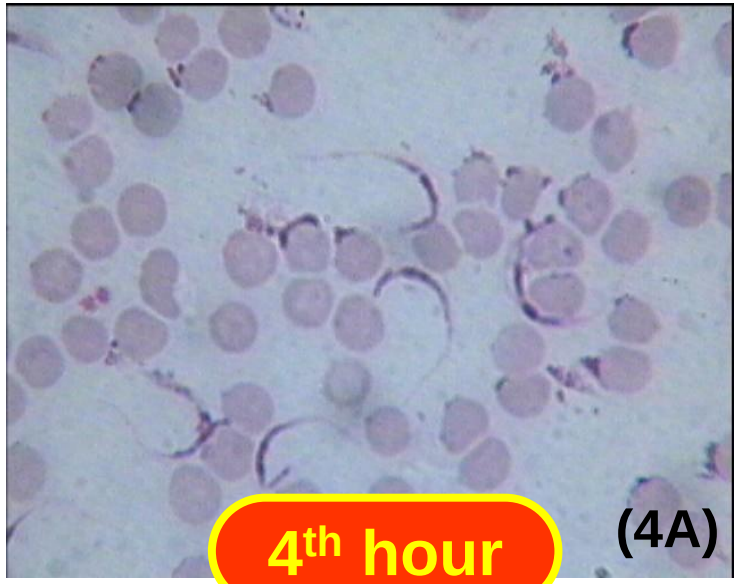


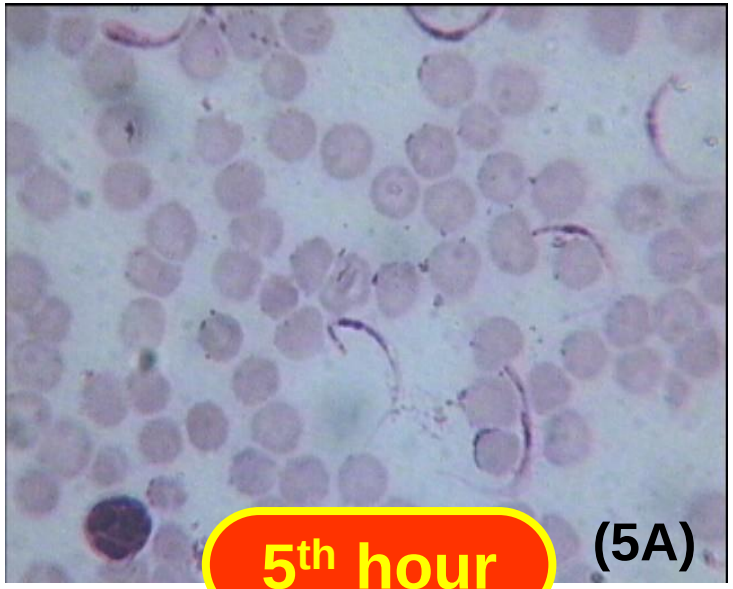
(2B)

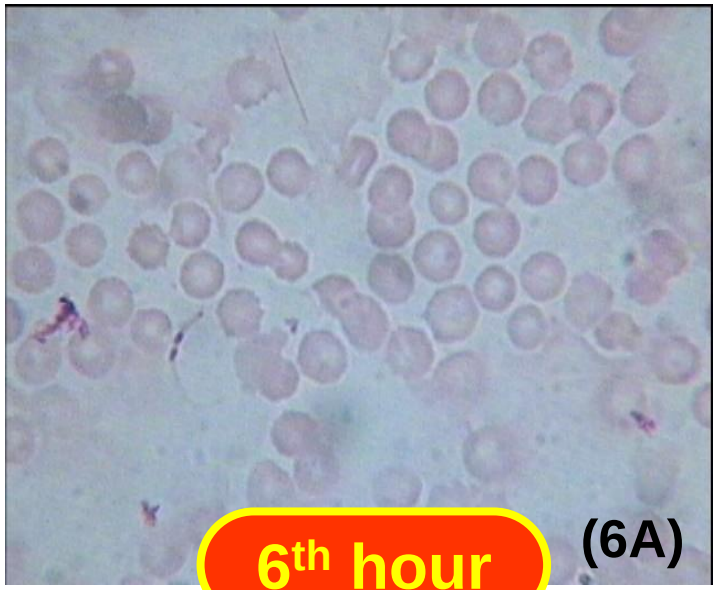


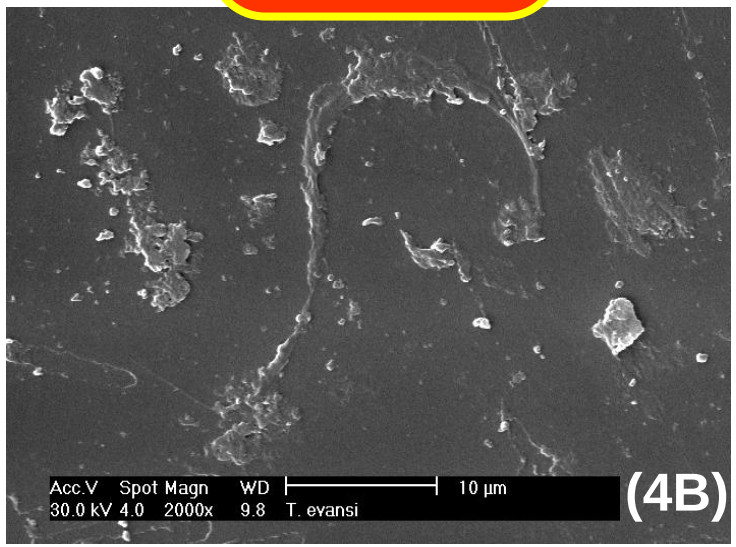
(3B)

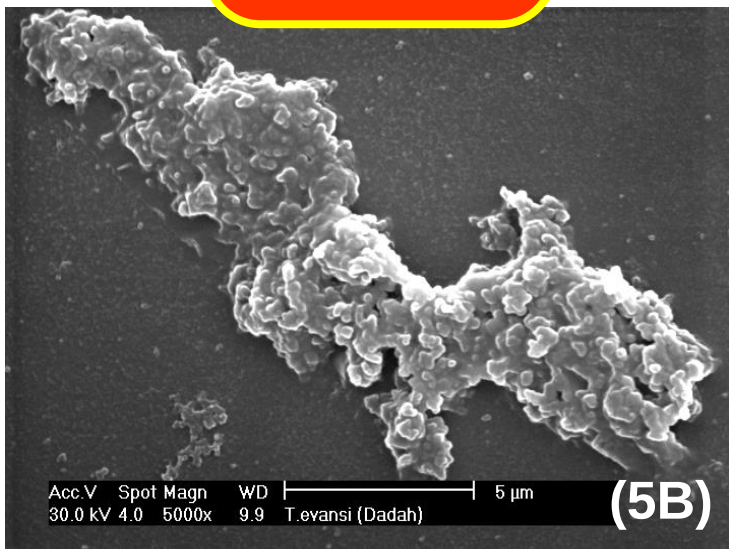
Parasite Growth in Berenil-Treated Group (POS)

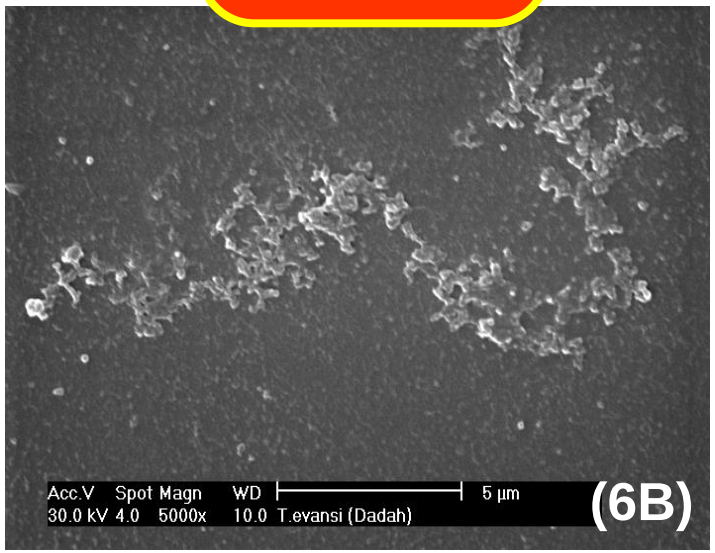




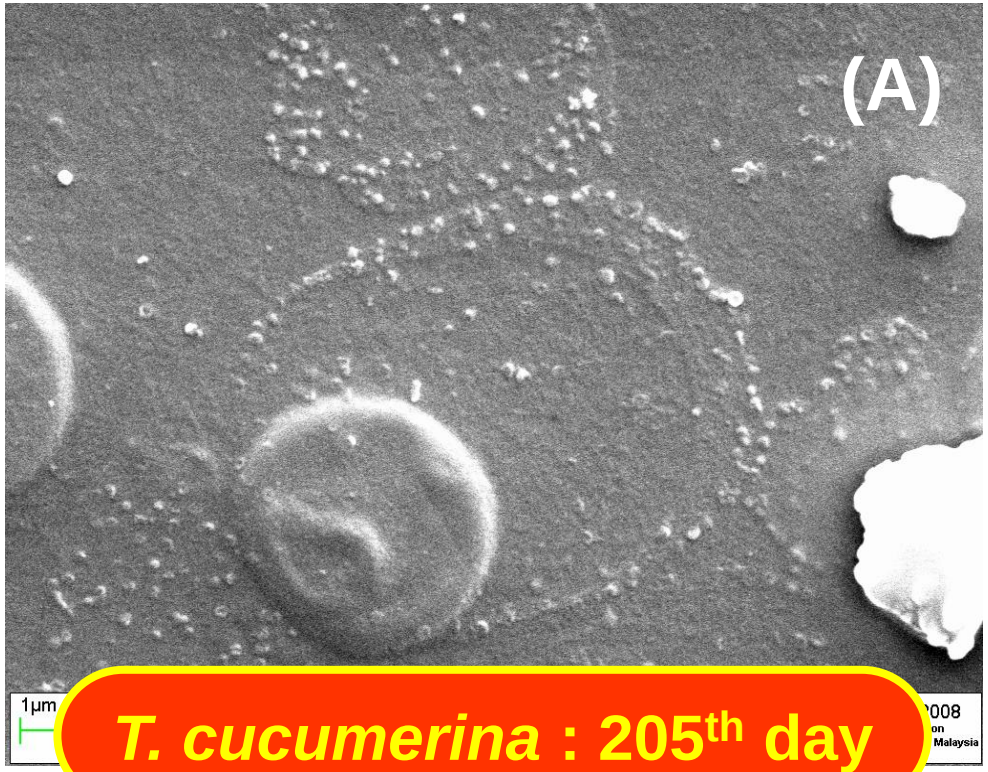


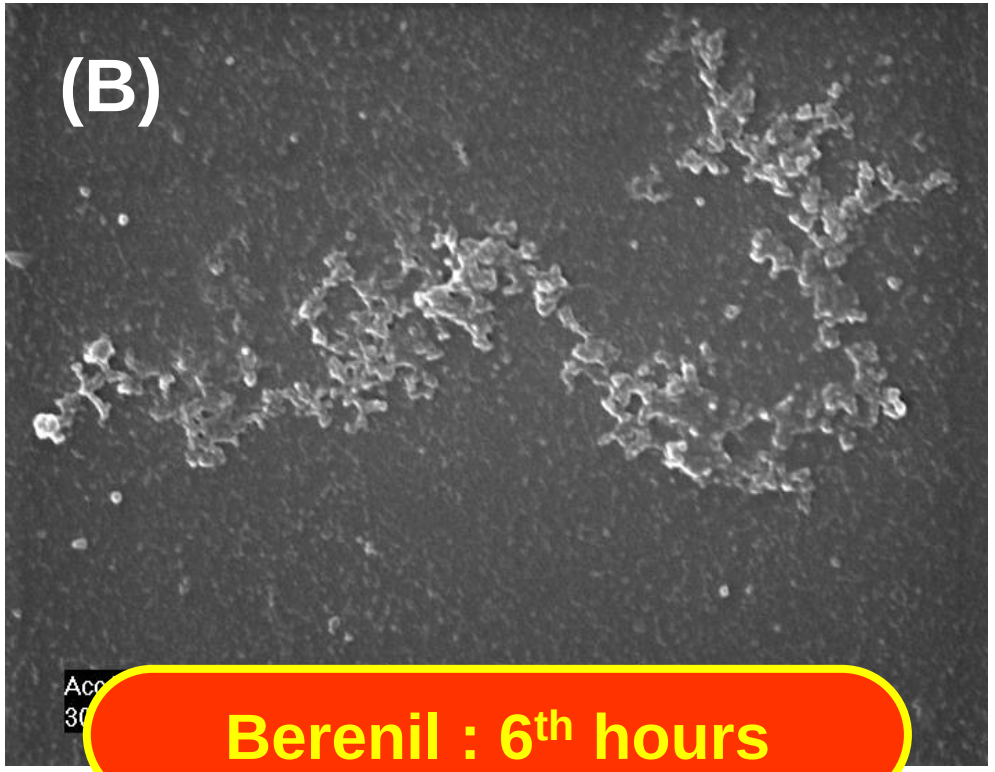






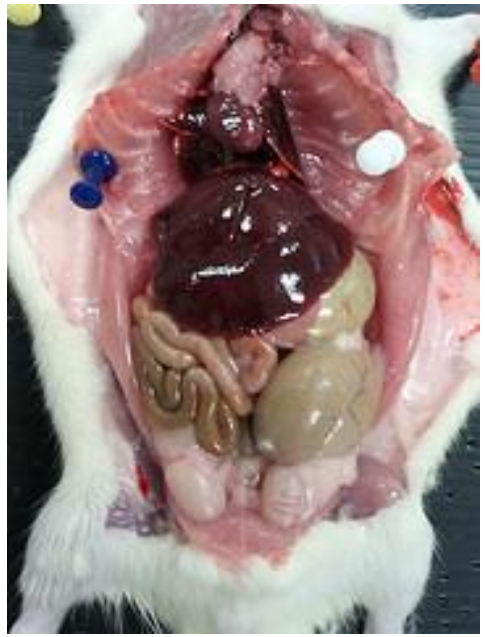
T. cucumerina VS Berenil





Scanning electron micrograph showed the morphological changes of *T. evansi* in PRE14 mice (0.2 mL 100 mg/kg bw *T. cucumerina* aqueous-extract) on 205th day post infection (A) and in POS mice at 6th hours post treatment (0.01mL 3.5 mg/kg bw Berenil) (B) as observed under x5000 magnification of SEM

Biochemical Test For Toxicity Assessment



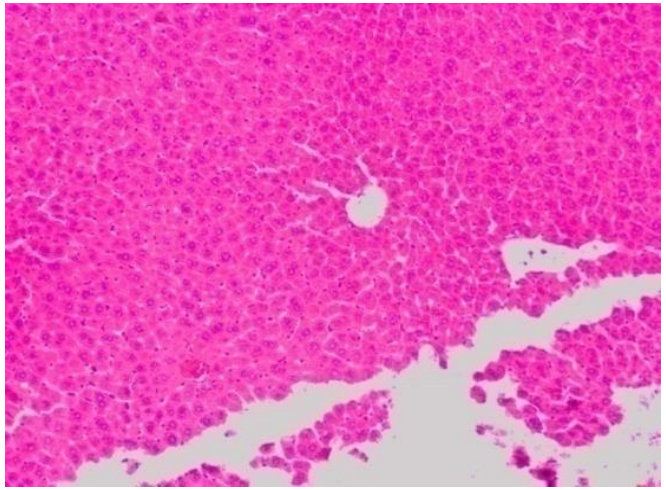
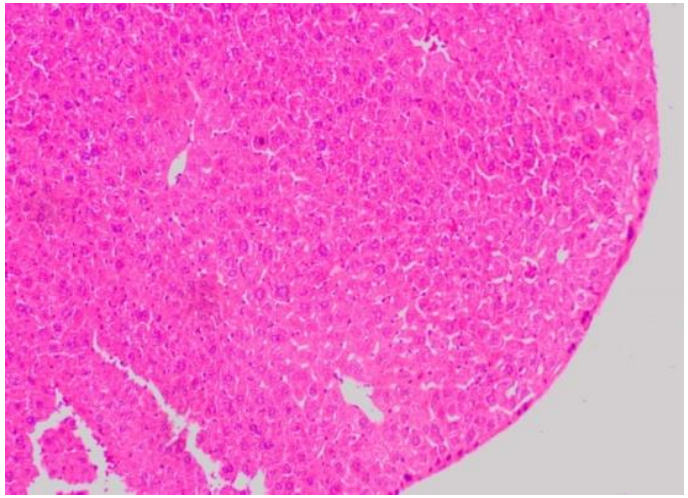
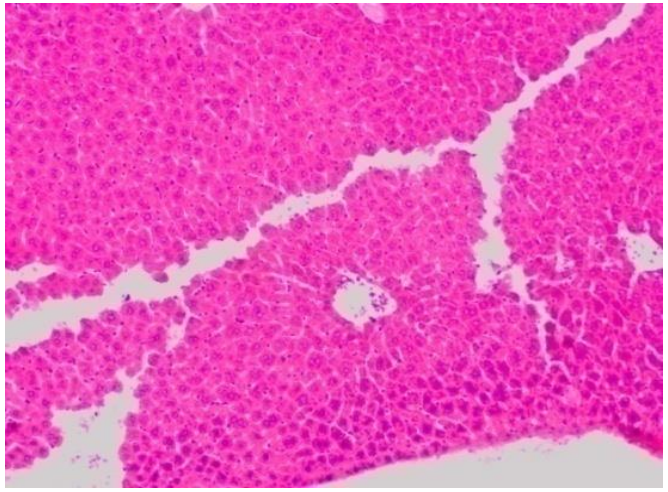
Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	40 – 93	IU/L
AST (*)	133.13 ± 2.04	125.93 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	92 – 206	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	61.46 ± 2.46	58.32 ± 2.97	54 – 115	IU/L
STP (*)	6.12 ± 2.32	7.21 ± 3.81	7.93 ± 2.01	8.83 ± 3.90	6.40 ± 1.01	6.80 ± 3.06	5.8 – 9.5	g/dL

TA : Sub-acute regime – Daily treatment (28 days)
TB : Sub-acute regime – Daily treatment (28 days) 2 hours post-infection
TC : Sub-chronic regime – Daily treatment (90 days)
TD : Sub-chronic regime – Daily treatment (90 days) 2 hours post-infection
CN : Control regime – Normal mice without infection and treatment
CI : Control regime – Infected mice on D0
ALT : Alanine aminotransferase
AST : Aspartate transaminase
ALP : Alkaline phosphatase
STP : Serum total protein

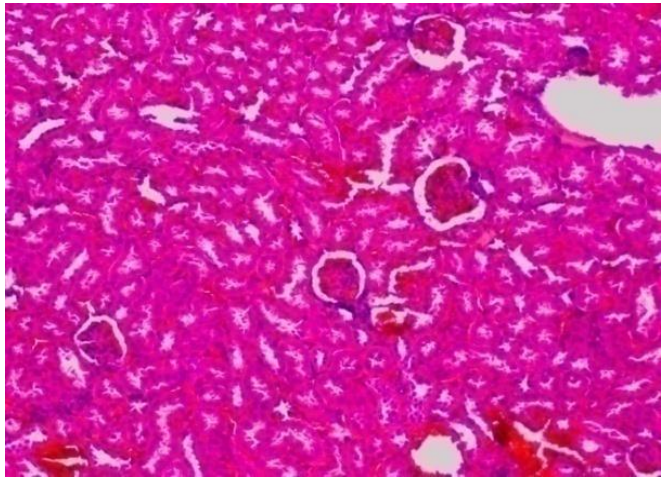
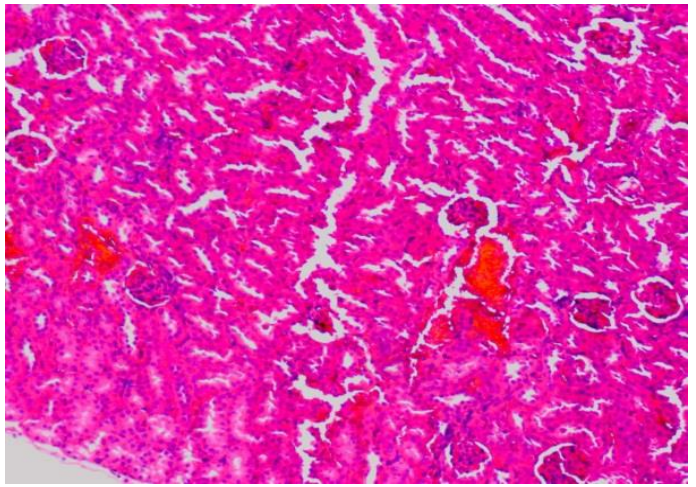
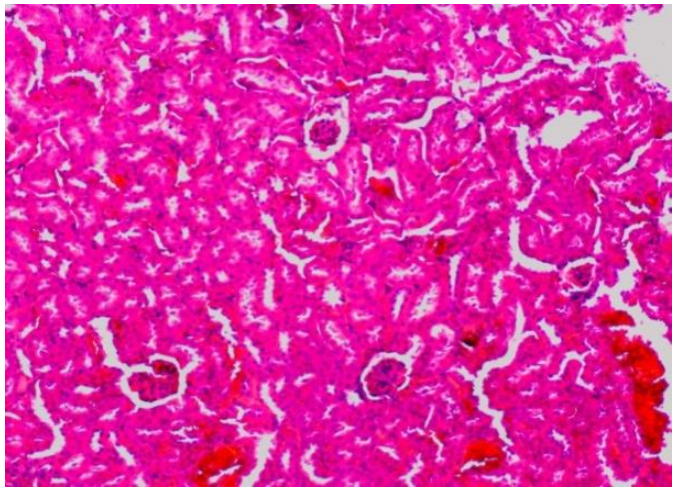
(*) ***All values were expressed as mean ± standard deviation (sd)***

Organ Histology For Toxicity Assessment

Liver

Kidney

Treatment (Acute) ***Treatment (Sub-acute)*** ***Control***

CONCLUSIONS

Hypothesis

- Tryptophol and stochastic genetic modification of VSA is still the best ‘weapon’ for *T. evansi* survival (Iniguez-Martinez et al. 2009).

- New wave of infection → mice is susceptible to infection (Kurup and Rajamohan 2011)

- The action of pheniprazine ($C_9H_{14}N$) molecule in *T. cucumerina* against –thiol group of parasite enzymes in which crucial for parasite proliferation (Ma et al. 2012).

- Bioactive compound of bivittoside in *T. cucumerina* inhibited the important enzymes for the stability of the redox reaction in tissues protozoan cells such as alcohol dehydrogenase, cysteine proteinase and thioredoxin reductase (Zhao et al. 2010)

Suggestions

Various solvents of *T. cucumerina* extract

Mechanism of action

In-vitro anti-trypanosomal screening

Concentration- & time-dependant alteration

Clinical & molecular approaches

Screening against *T. cruzi* and *T. brucei*

Absolute Hypothesis

EAT SNAKE GOURD..!

NO HARM TO EAT AS MUCH AS YOU CAN

Absolute Hypothesis

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Thank You

Rationale Of The Study

Reliability of Anti-Trypanosomial Drugs

- Resistant issues in India, Thailand & Indonesia
- Unaffordable → expensive in certain regions
- Wrong dosage & concentration → side effects

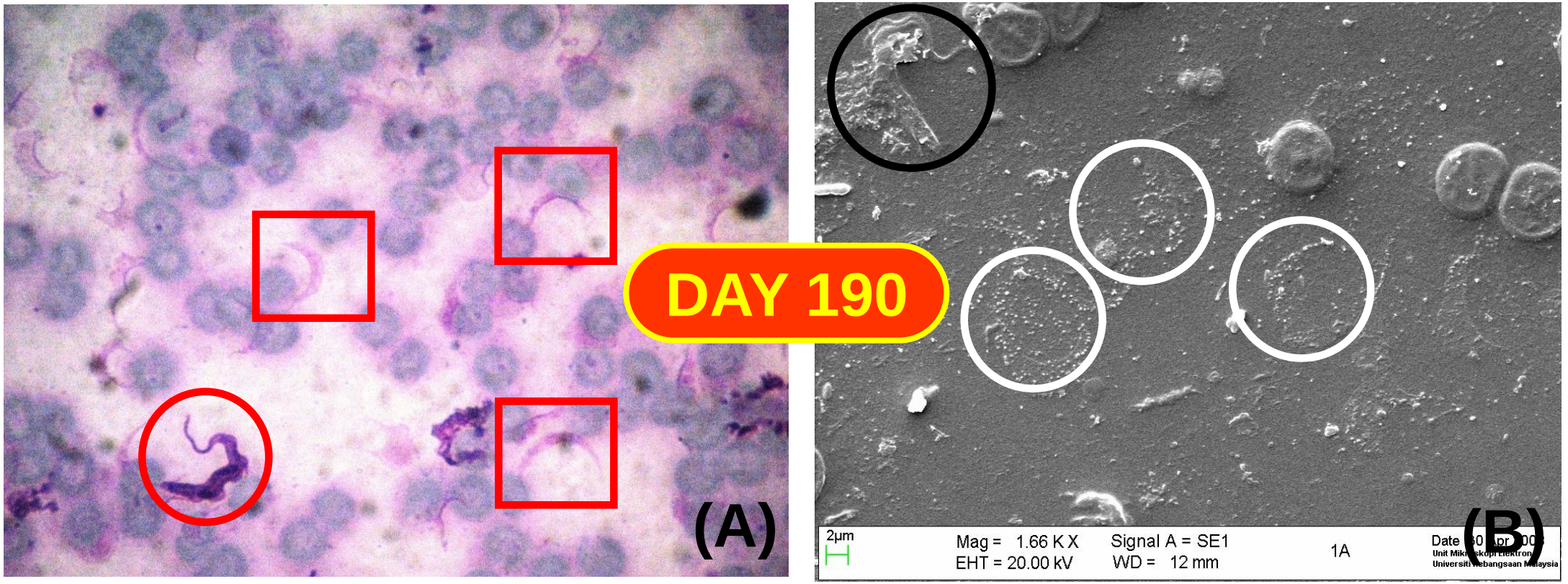
Economic Growth & Biotechnology Sector

- Biotechnology → main focus in the next decade
- Snake gourd → consumable & easily manipulated
- AHT & Surra → influenced productivity of human & livestock

Current Issues of *T. evansi*

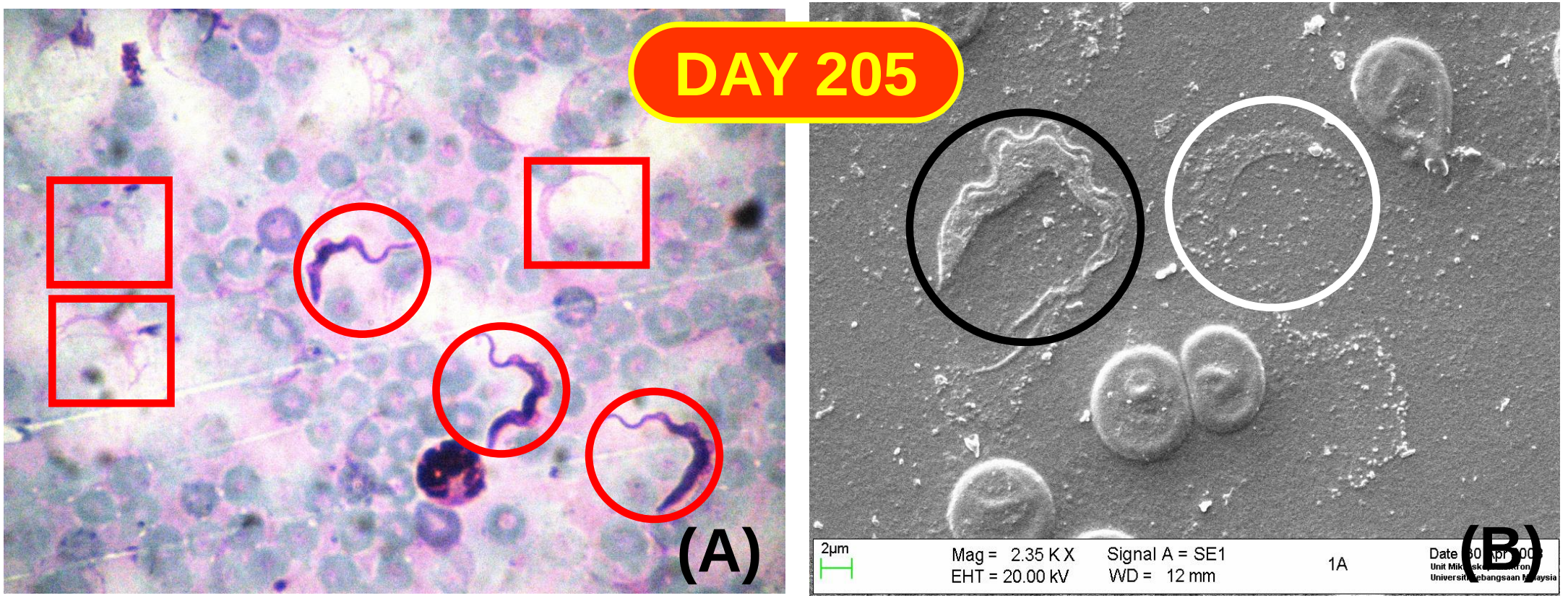
- Trans-host boundary : animal → human (Assam India 2008)

Parasite Survival In PRE14 Mice Group : 190th Day



Reemerged of *T. evansi* which survived in PRE14 group mice on day 190 due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as observed under x100 magnification of light microscope (A) and x1600 magnification of SEM (Leo 1450VP, Japan) (B).

Parasite Survival In PRE14 Mice Group : 205th Day



Reemerged of *T. evansi* which survived in PRE14 group mice on day 205 due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as observed under x100 magnification of light microscope (A) and x2300 magnification of SEM (Leo 1450VP, Japan) (B). Later the mice died on day 211

‘Variable Surface Glycoprotein’ (VSG)

- Survival factor of *Trypanosoma* spp. in the infected host
- High density layer on the parasite cell membrane
- Contained 1 x 10^9 similar & uniformed glycoprotein molecules expressed by VSG-Trypanosome gene
- Protect the parasite from being identified/action of the host immune system
- Similar & uniformed glycoprotein molecule → only end region of `N-terminal loops’ structure (300-500 amino acid structures) can be identified by the host immune systems → specific antibody-antigen mechanisms

‘Variable Surface Glikoprotein’ (VSG) – cont.

- When the end region of `N-terminal loops’ structure being identified by the host immune systems → VSG-stochastic genetic modification’ of the parasite plays the role.
- VSG stochastic genetic modification = periodic changes of antigenic variation → the structures & characteristics of parasite cell membrane was modified whenever confronted with the host’s specific immune system which may varies.
- Periodic changes of antigenic variation → changes in parasitemia waves → longer survival time of the parasite → chronic infection on host

Survival Pattern of the Trypanosomiasis Infected-Host Due to VSG-Stochastic Genetic Modification Phenomenon