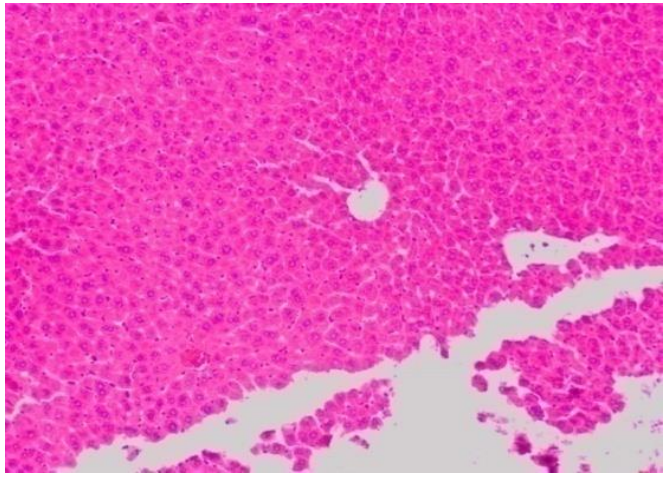
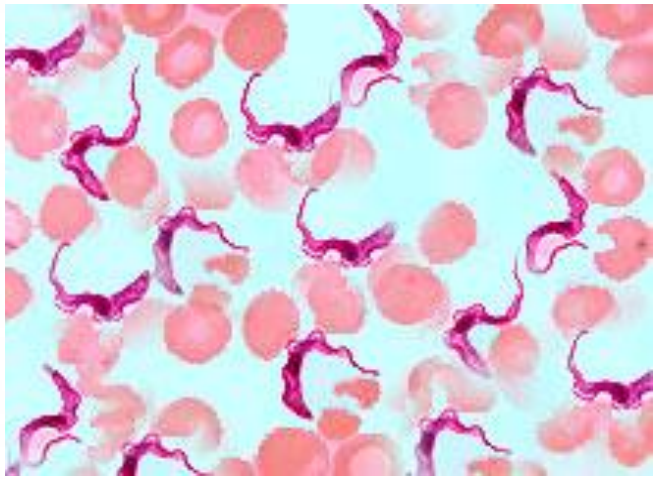
IN-VIVO ANTIPARASITIC ASSESSMENT AND TOXICITY EVALUATION OF *Curcuma longa* AGAINST THE GROWTH AND SURVIVAL OF *Trypanosoma evansi* IN MICE





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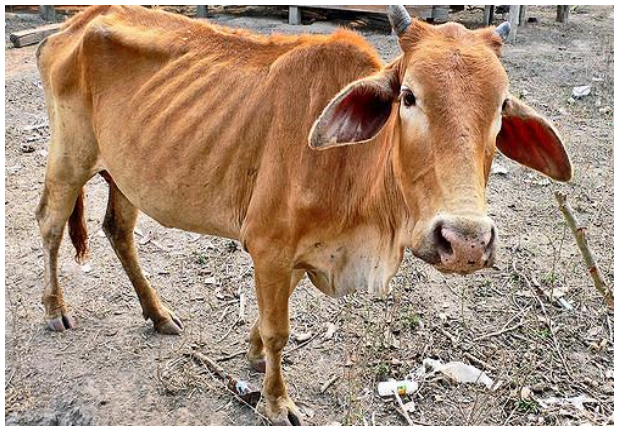
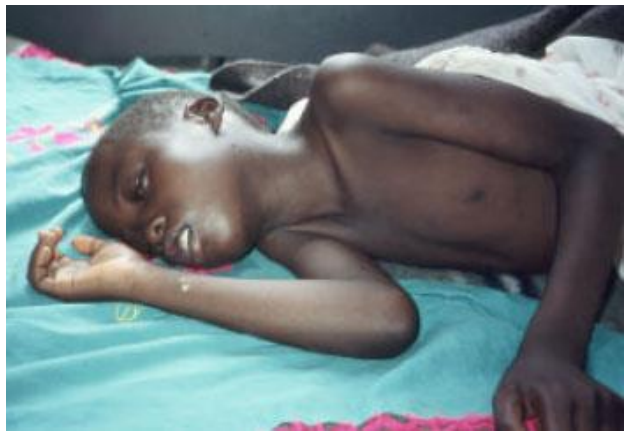
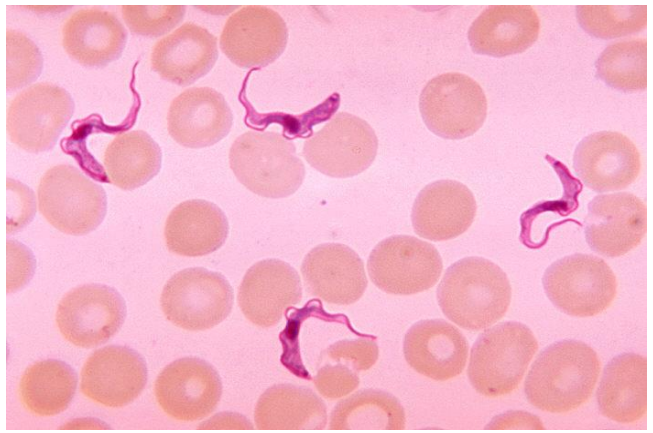
INTRODUCTION

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*

Horsefly /*Tabanus striatus*

Tsetse fly / *Glossina morsitans*

Horn fly / *Haematobia* spp.

Muscidae fly / *Stomoxys spp.*

Another Possible Vectors of *Trypanosoma evansi*

Triatominae bug / *Triatoma* spp.

Argasidae tick / *Ornithordorus* spp.

Buffalo leech / *Hiruninaria manillensis*

Vampire bat / *Desmodus rotundus*

Documented AHT (Philippe *et al.* 2013)

No	Location	Species / Subspecies	Date	Identification Method	Fever	Treatment	Outcomes
1	Ghana	*T. vivax*	1917	Morphology	ND	ND	ND
2	Pasteur Inst.	*T. b. brucei*	1930	Morphology	ND	ND	ND
3	Congo	*T. b. brucei*	1947	Morphology	Present	None	Self-cured
4	Ethiopia	*T. b. brucei*	1987	Morphology	ND	ND	Cured
5	Ghana	*T. b. brucei*	2003	PCR	Present	None	Self-cured
6	Ivory Coast	*T. congolense*	1998	PCR	Present	Pentamidine	Cured
7	India	*T. evansi*	1977	Morphology	Present	Atoxyl	Cured
8	Sri Lanka	*T. evansi*	1999	Morphology	Present	None	Self-cured
9	India	*T. evansi*	2004	PCR	Present	Suramin	Cured
10	India	*T. evansi*	2005	Morphology	Present	None	Death
11	Egypt	*T. evansi*	2010	Morphology	Present	ND	Cured
12	Malaysia	*T. lewisi*	1933	Morphology	Present	None	Self-cured
13	India	*T. lewisi*	1974	Morphology	Present	None	Self-cured
14	India	*T. lewisi*	1974	Morphology	Present	None	Self-cured
15	Gambia	*T. lewisi*-like	2003	PCR	Present	Melarsoprol	Cured
16	Thailand	*T. lewisi*-like	2003	PCR	Present	Antibiotic	Cured
17	India	*T. lewisi*	2006	Morphology	Present	None	Self-cured
18	India	*T. lewisi*	2007	PCR	Present	Suramin	Death
19	India	*T. lewisi*	2010	PCR	Present	Pentamidin	Cured

Curcuma longa : The Testimonial

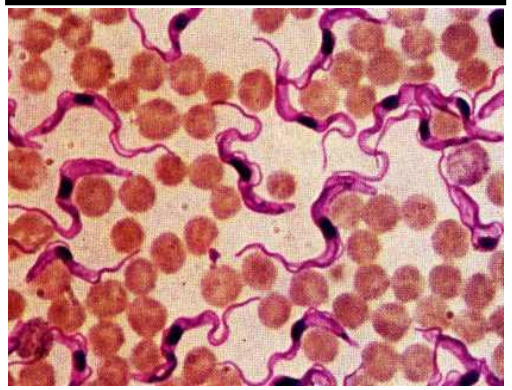
- Outstanding antiulcer element in rats (Rafatullah et al, 2009)
- In-vitro & in-vivo antiplasmodial activities (Almalki et al, 2017)
- Antifungal activity of turmeric oil (Apisariyakul et al, 2015)
- In-vitro & in-vivo anticancer elements (Kuttan et al, 2015)
- Antivenom property of essential oil (Ferriera et al, 2012)
- Antihelmintic activity of turmeric powder (Singh et al, 2017)

- Anti-MRSA activity of turmeric oil (Gupta et al, 2015)
- Antidiabetic activities on NIDDM rats (Srinivasan, 2008)
- Hepatoprotective activities (Arawwawala et al. 2010)
- Inhibitory effects on Influenza A virus (Dao et al, 2012)
- Antioxidant effects of essential oil (Avanco et al, 2017)
- Inhibit biofilms development (Packiavathy et al, 2014)

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 _Curcuma longa_ aqueous extract

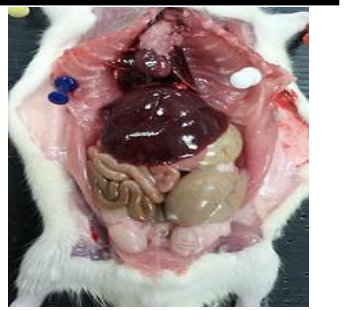
Giemsa blood slide for inhibition rate evaluation

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

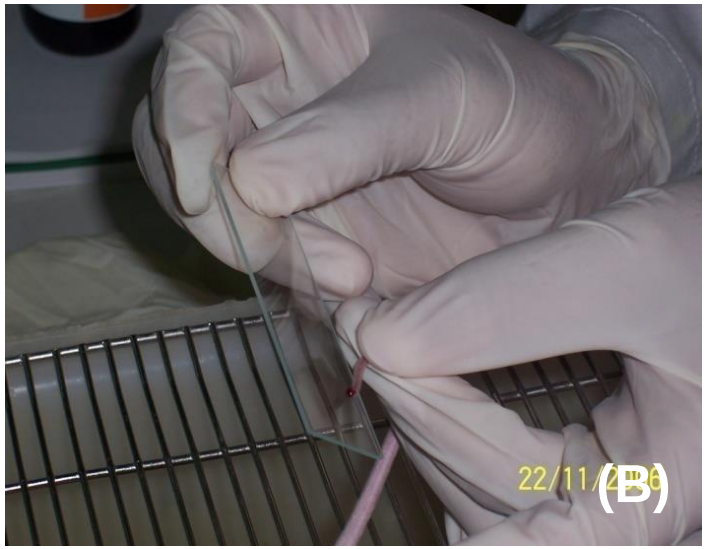


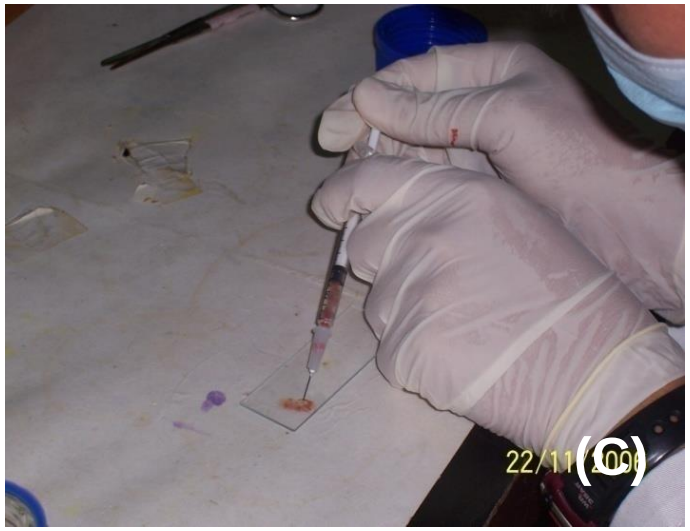
Experimental Design

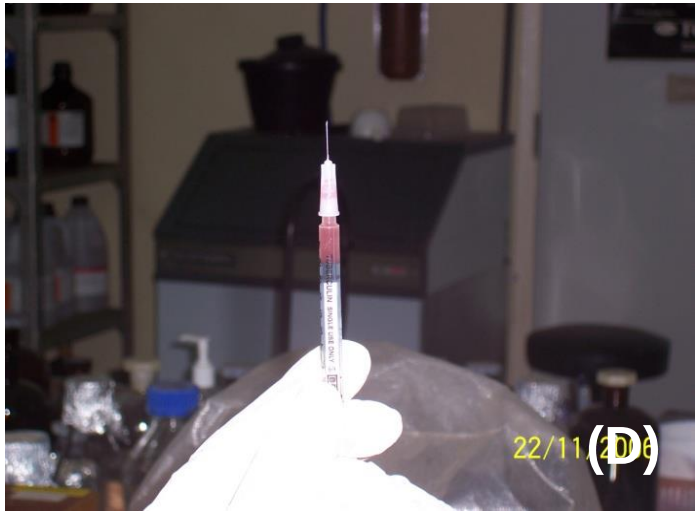
GROUP	REGIME	DESCRIPTION	*Curcuma longa* DOSAGE
TREATMENT	PREVENTIVE	14 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
		7 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
		3 days pre-infection	0.2 mL 100 mg/kg bw aqueous-extract
	CURATIVE	3 days post-infection	0.2 mL 100 mg/kg bw aqueous-extract
		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

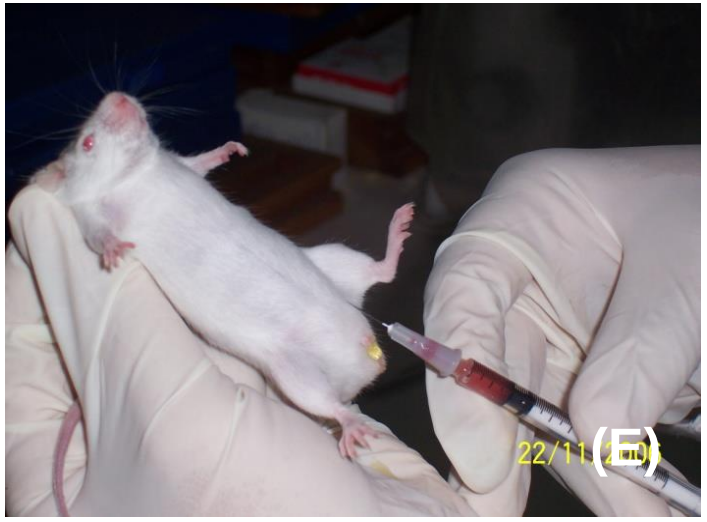
GROUP	REGIME	DESCRIPTION	CONTROL DOSAGE
CONTROL	POSITIVE	Berenil (Sigma-Aldrich KL)	0.01 mL 3.5 mg/kg bw
	NEGATIVE	0.9 % Normal Saline	0.1 mL 0.9 normal saline
	LETHAL INFECTION	Infection without treatment	5×10^3 *T. evansi* / mice (i.p.)

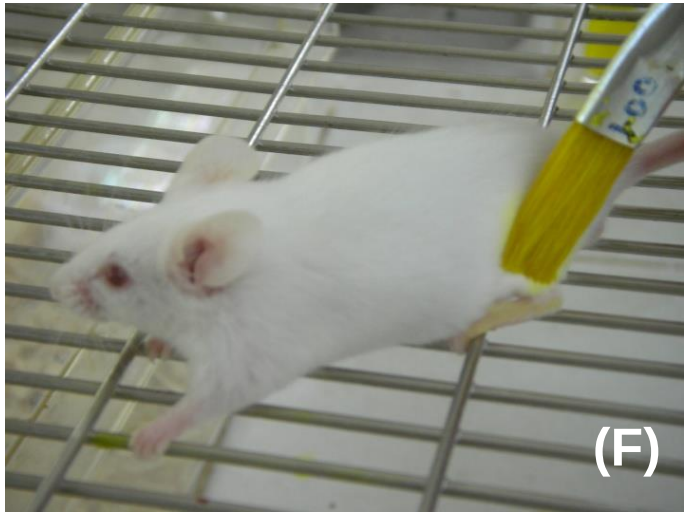
Parasite Administration And Animal Tagging



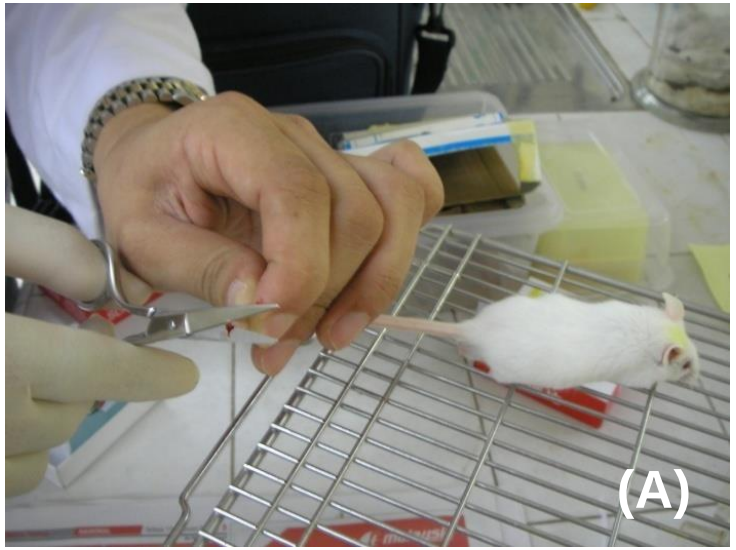


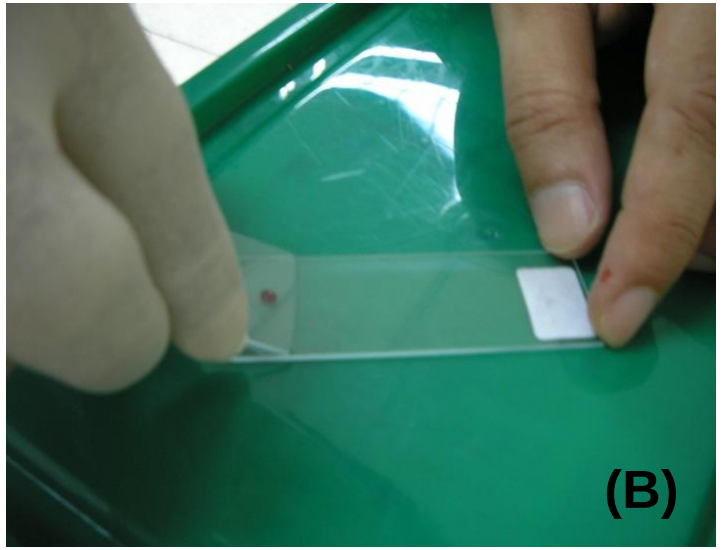


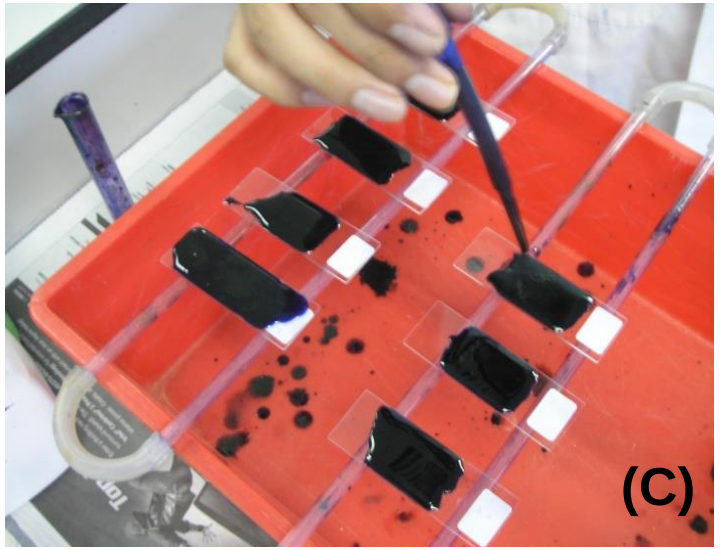


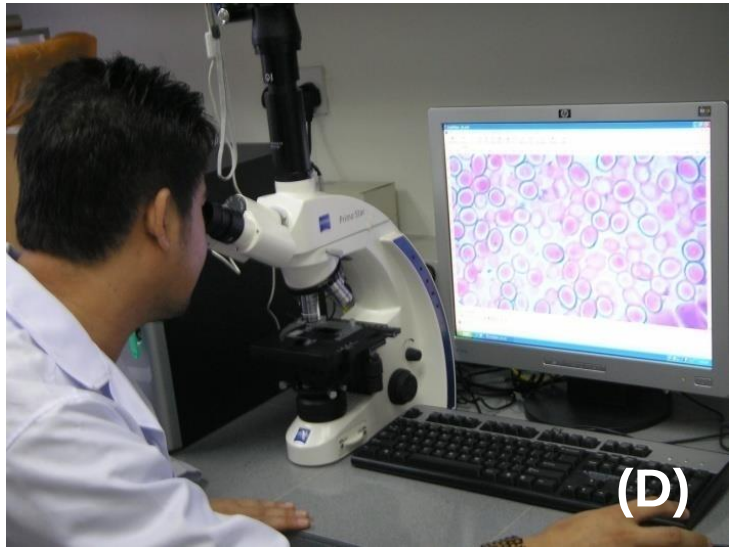


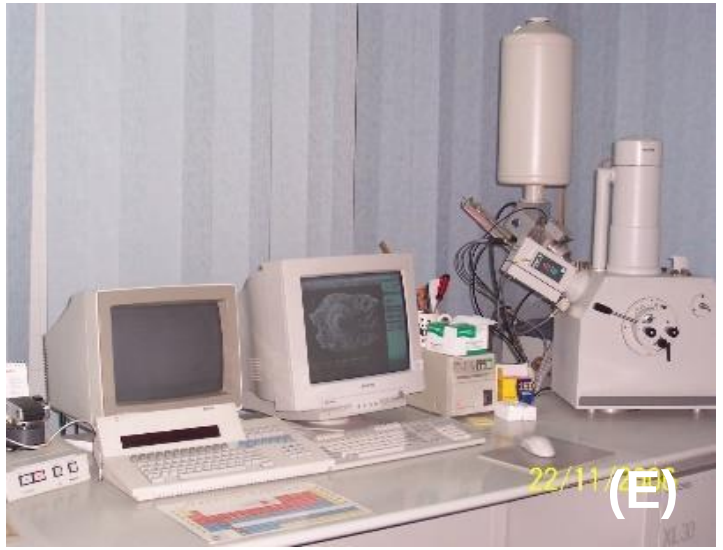
Giemsa Staining And Microscopic Observation

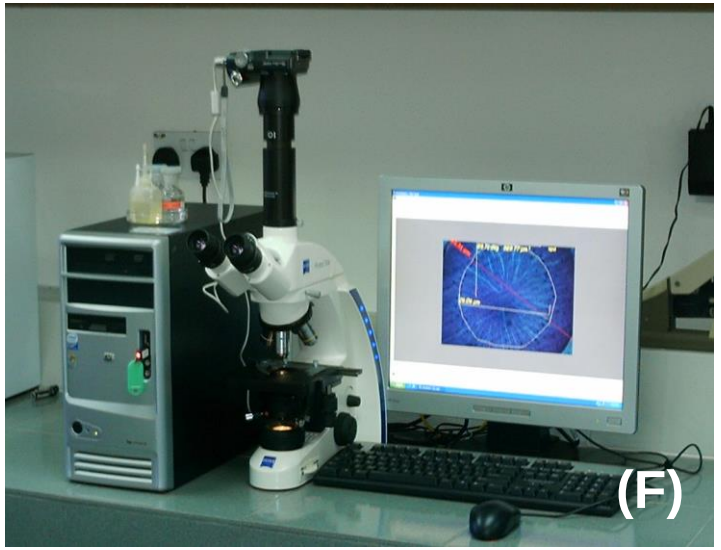




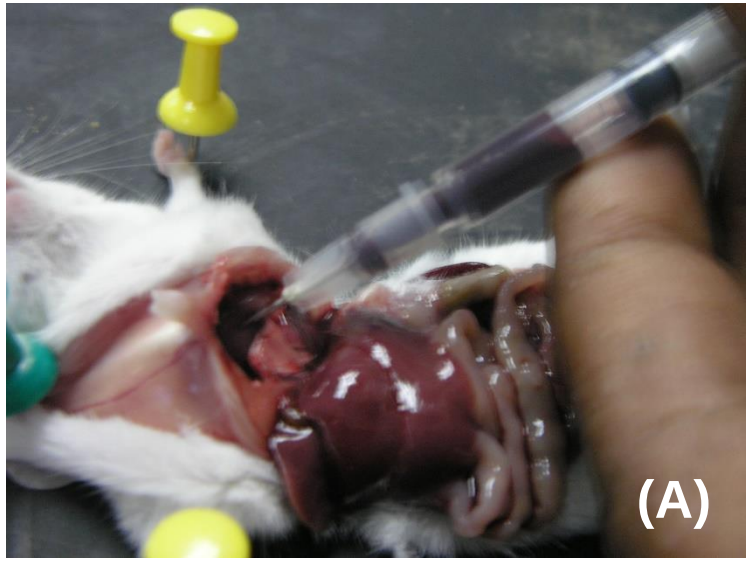


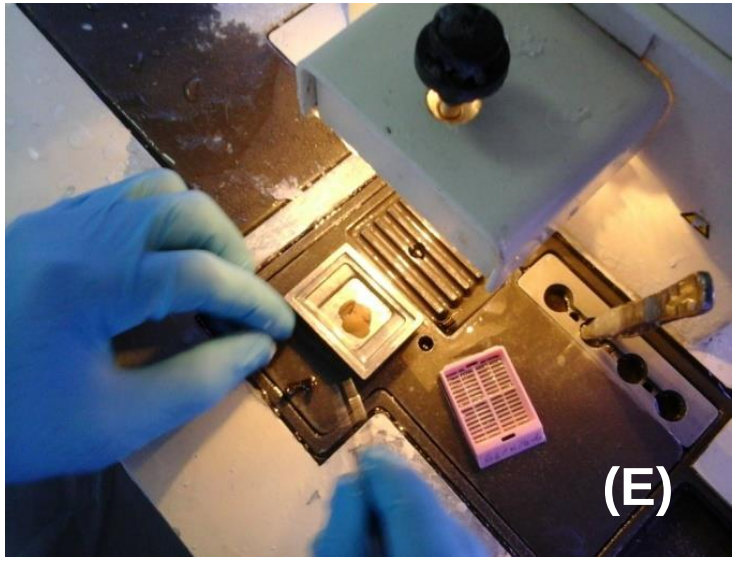






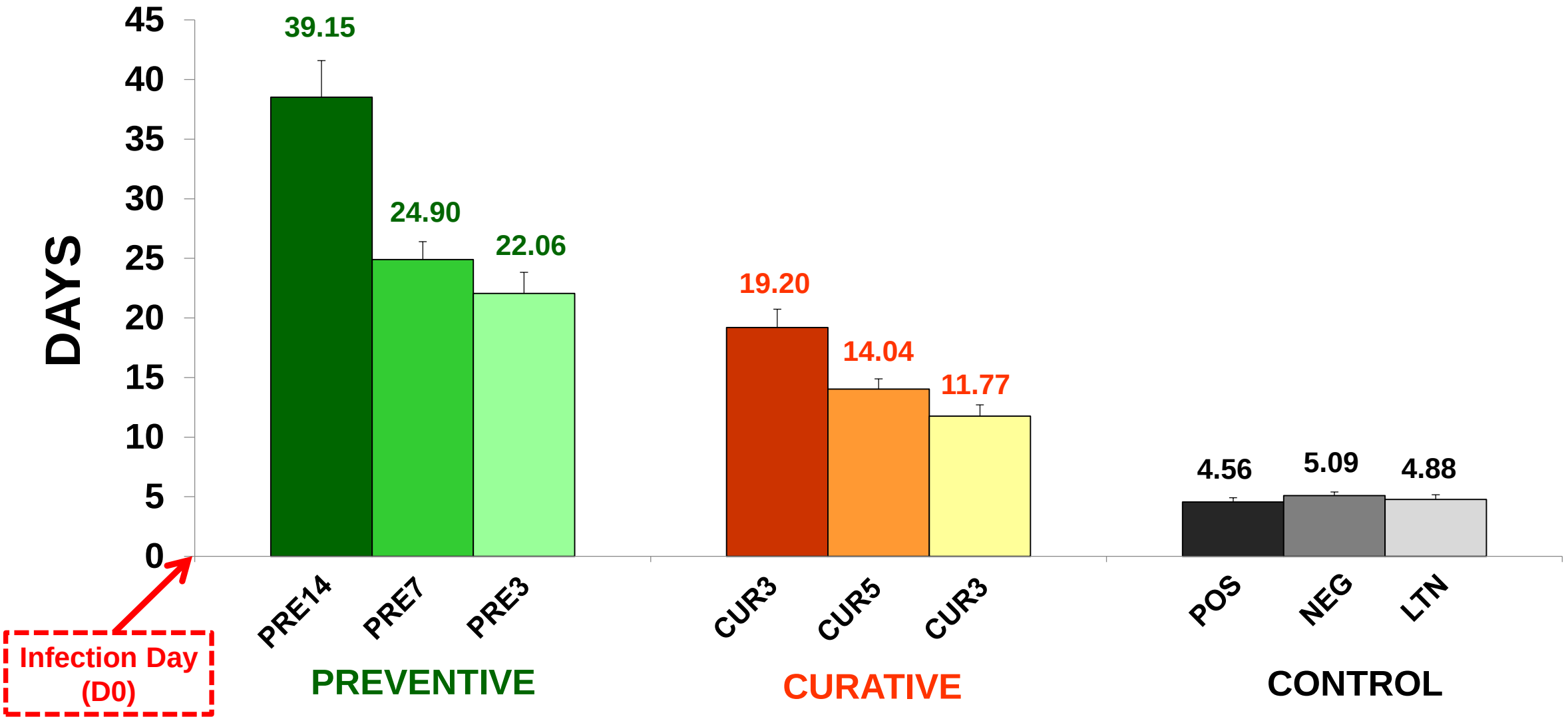
Biochemical Test And Histology Of Liver & Kidney



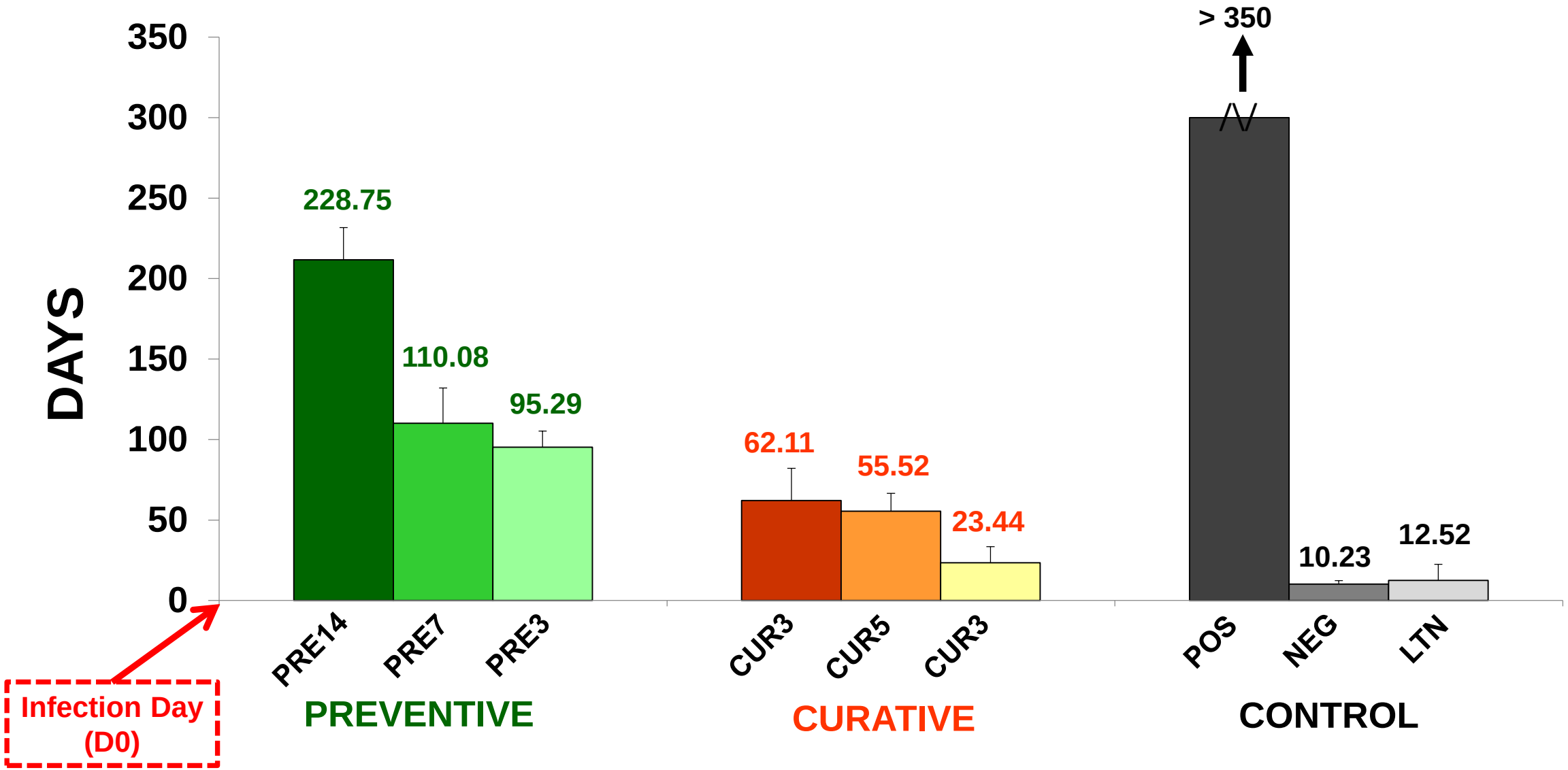


RESULTS & DISCUSSIONS

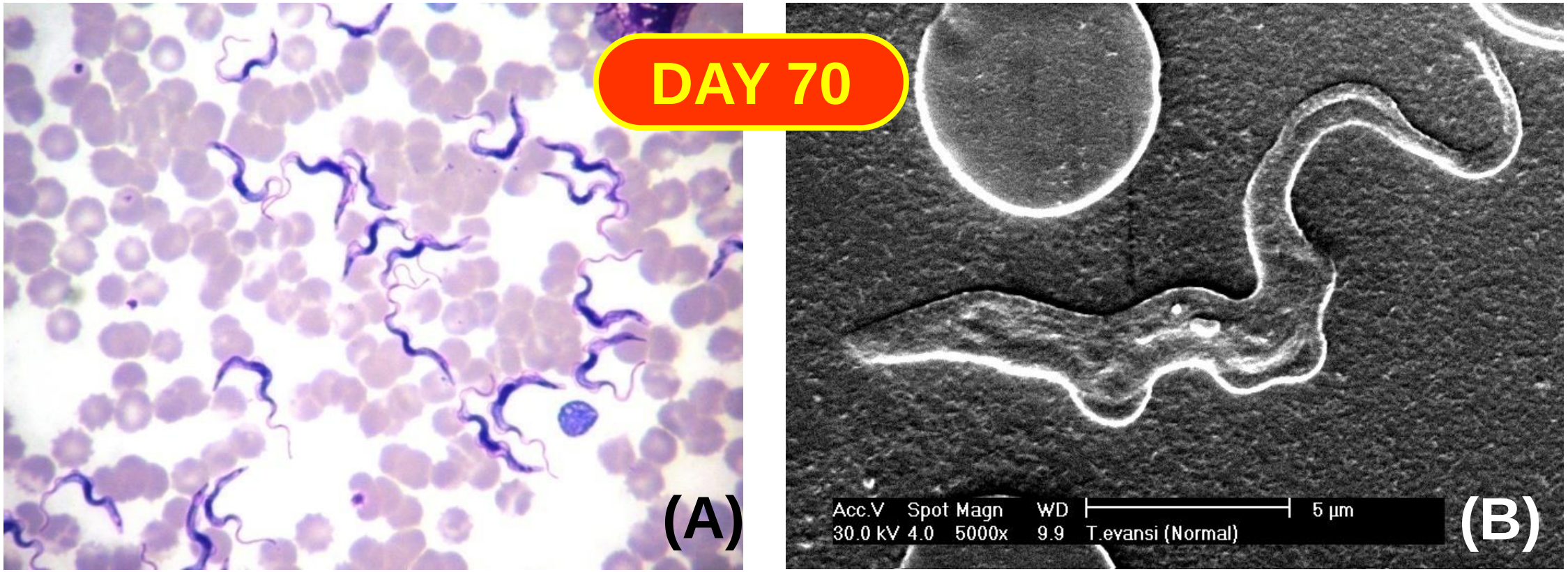
Parasite Pre-Patent Period (Day)



Mice Survival Time (Day)

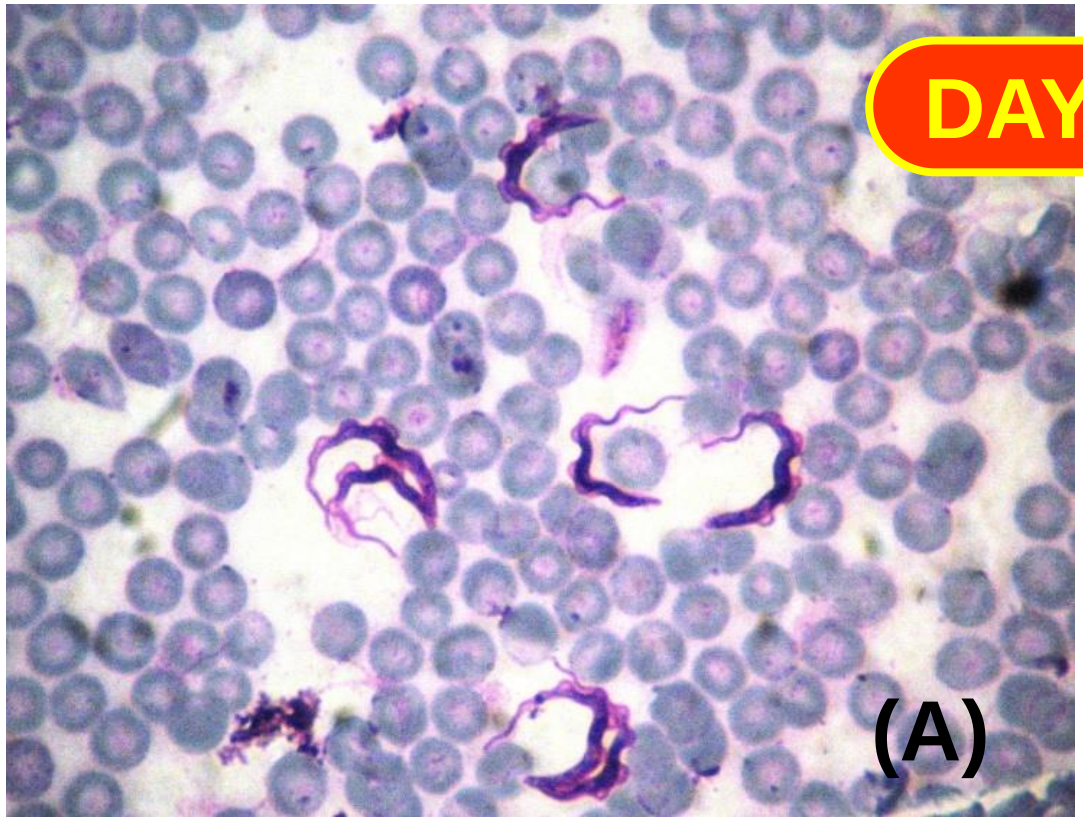


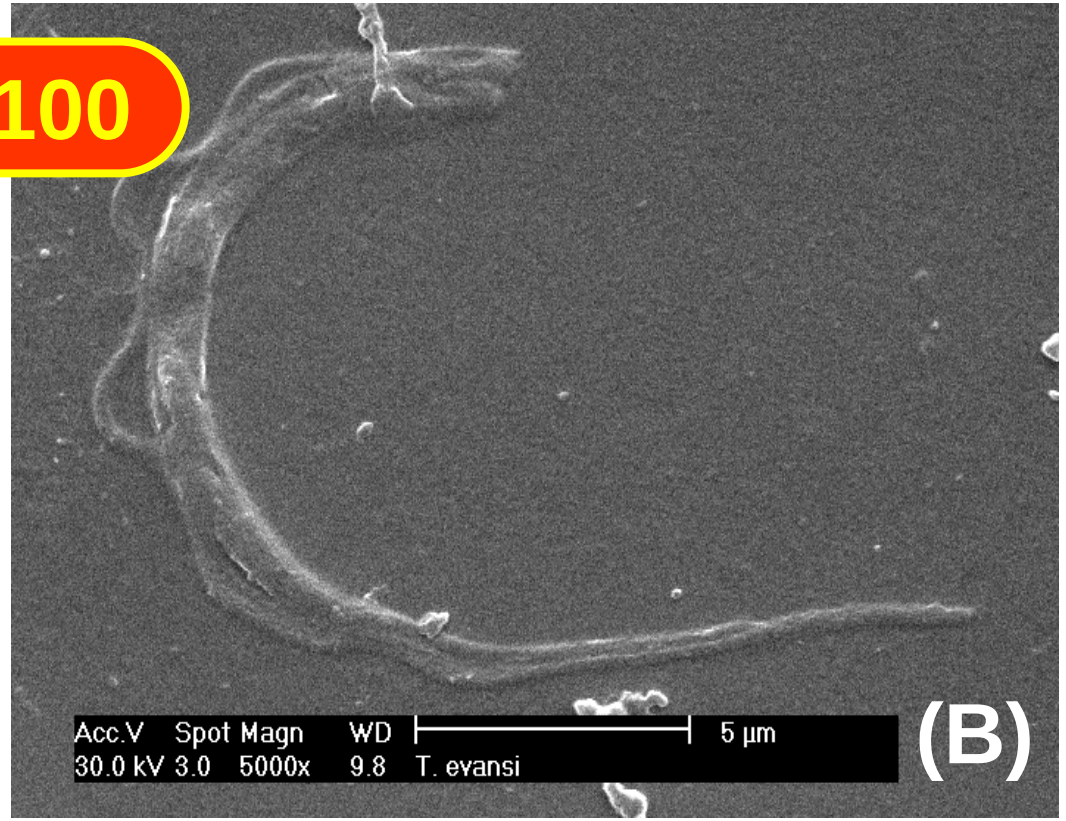
Parasite Survival In PRE14 Mice Group : 70th Day



Giemsa thin blood smear of the mice from PRE14 mice group taken on day 70 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

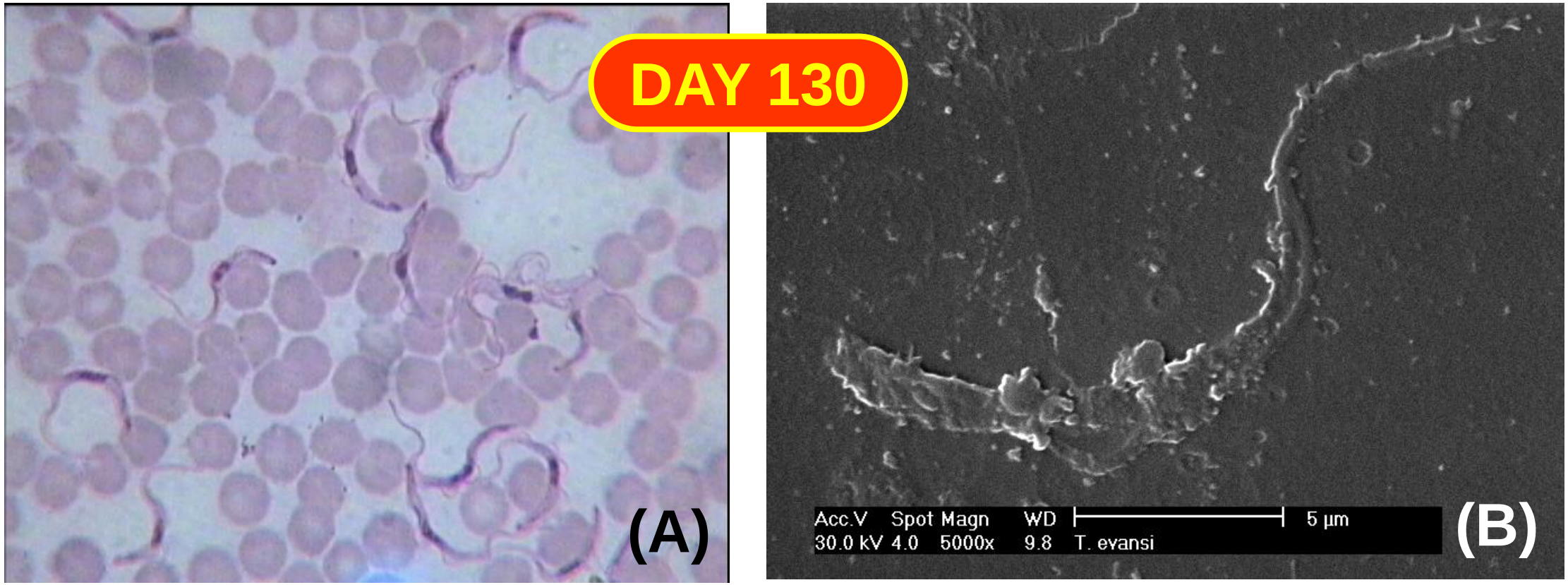
Parasite Survival In PRE14 Mice Group : 100th Day





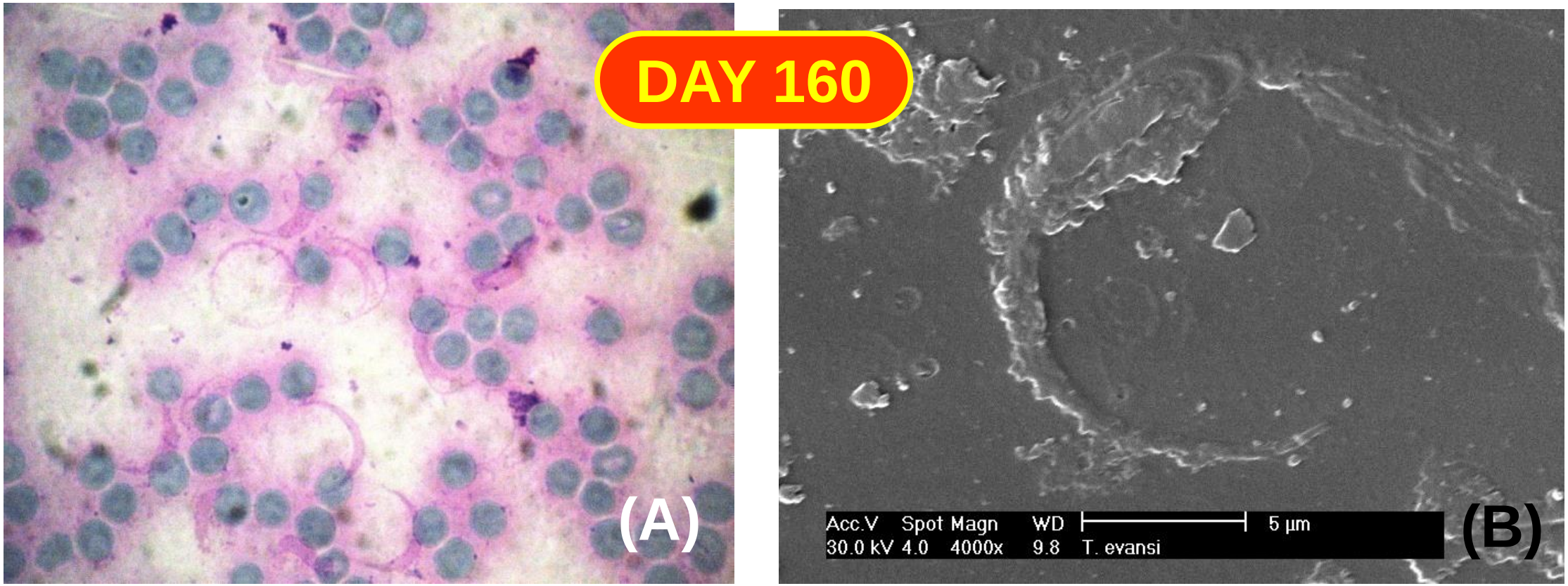
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 100 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

Parasite Survival In PRE14 Mice Group : 130th Day



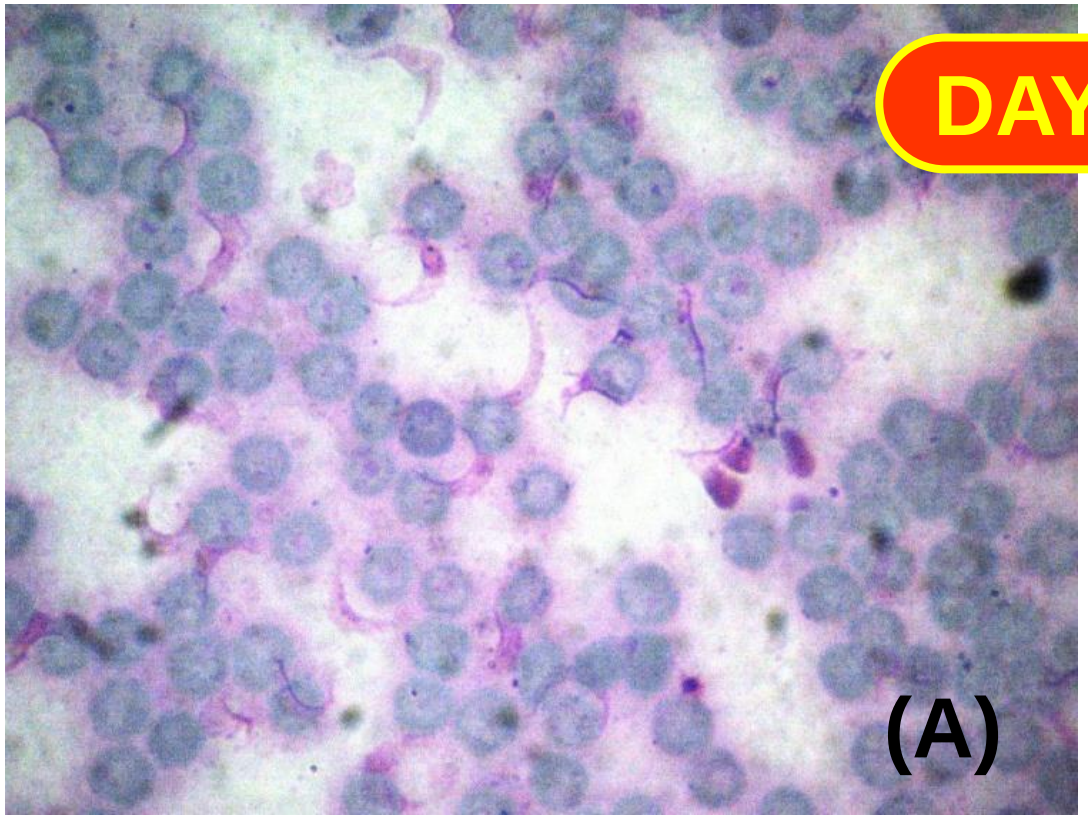
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 130 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

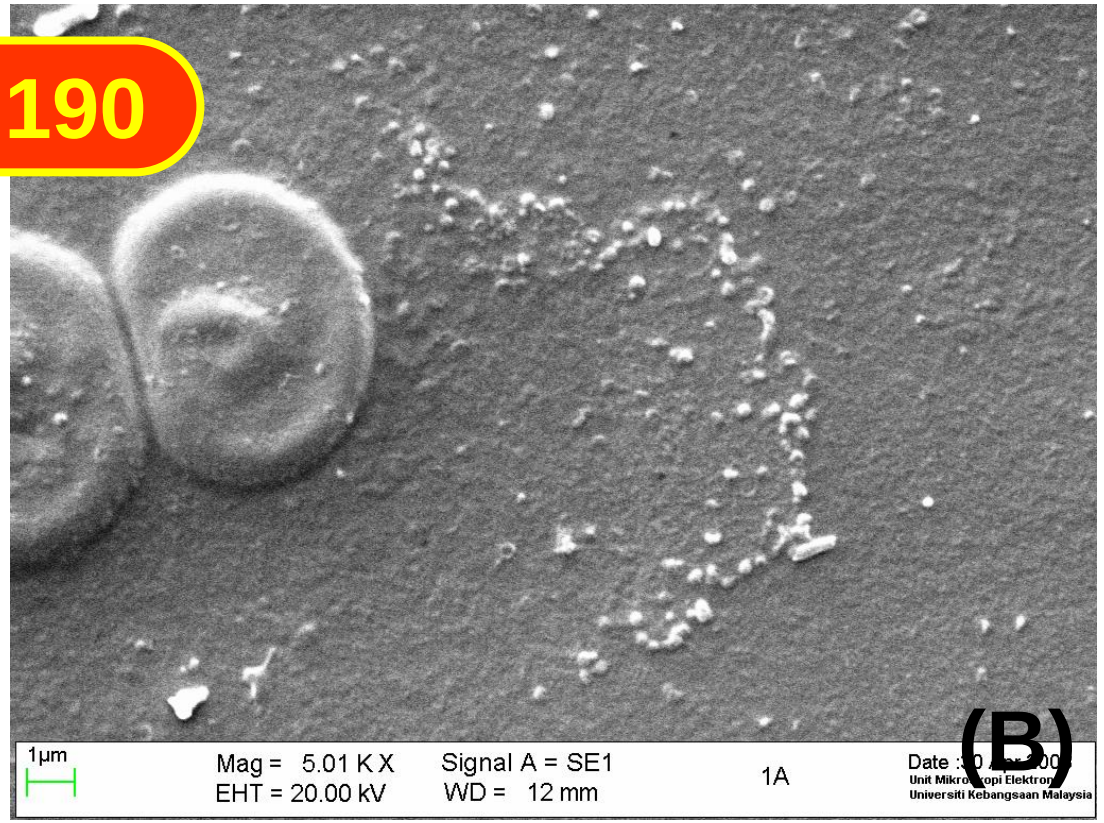
Parasite Survival In PRE14 Mice Group : 160th Day



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 x4000 magnification of SEM (Phillips XL30, UK) (B)

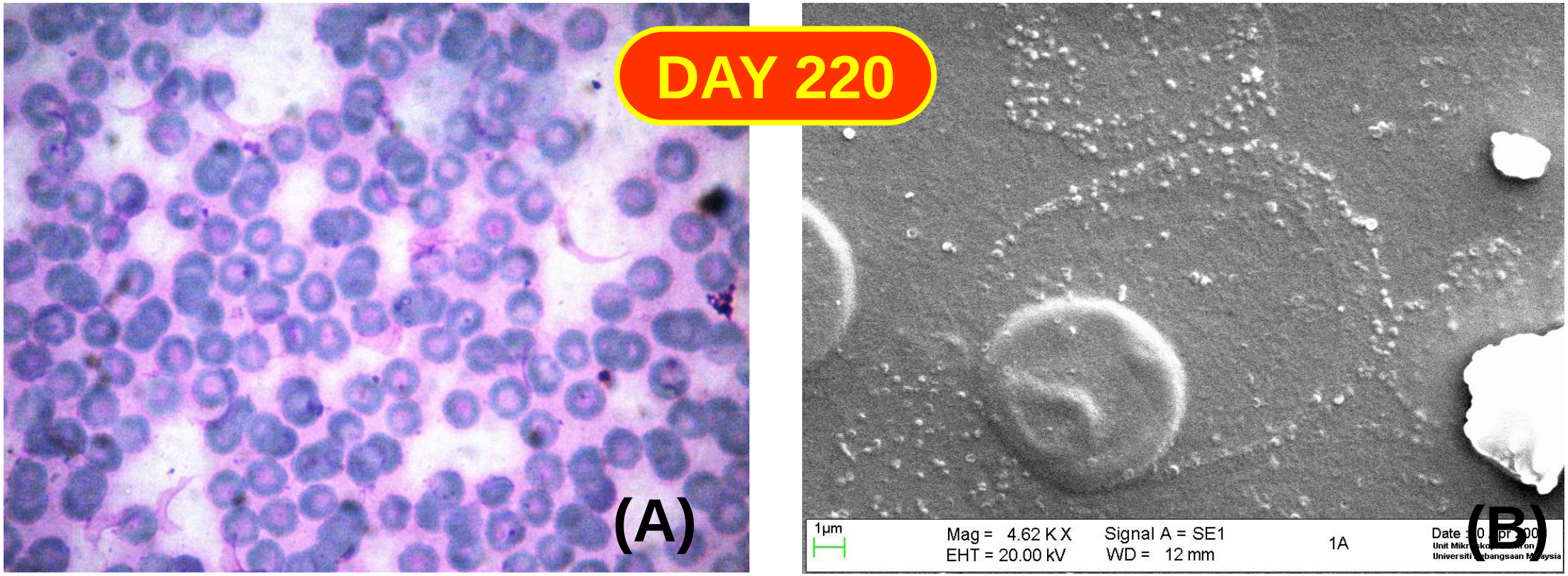
Parasite Survival In PRE14 Mice Group : 190th Day





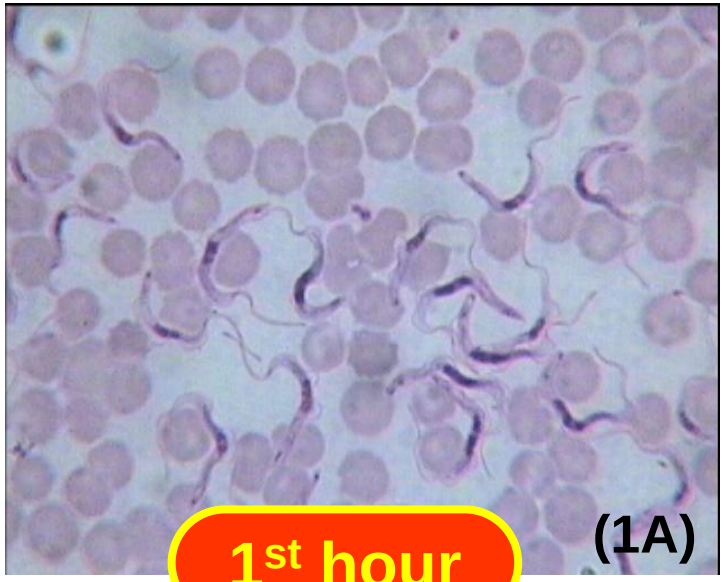
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 190 post-infection as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Survival In PRE14 Mice Group : 220th Day



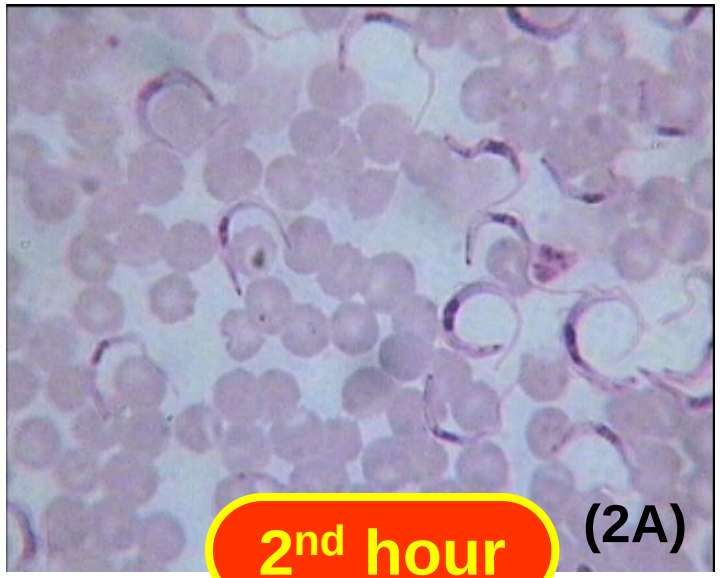
Giemsa thin blood smear of the mice from PRE14 mice group taken on day 220 post-infection as observed under x100 magnification of light microscope (A) and x4600 magnification of SEM (Leo 1450VP, Japan) (B)

Parasite Growth in Berenil-Treated Group (POS)



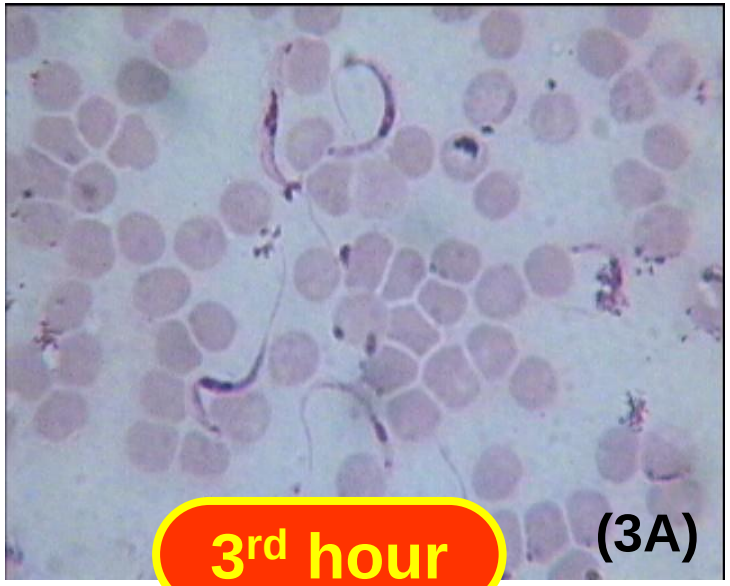
1st hour

(1A)



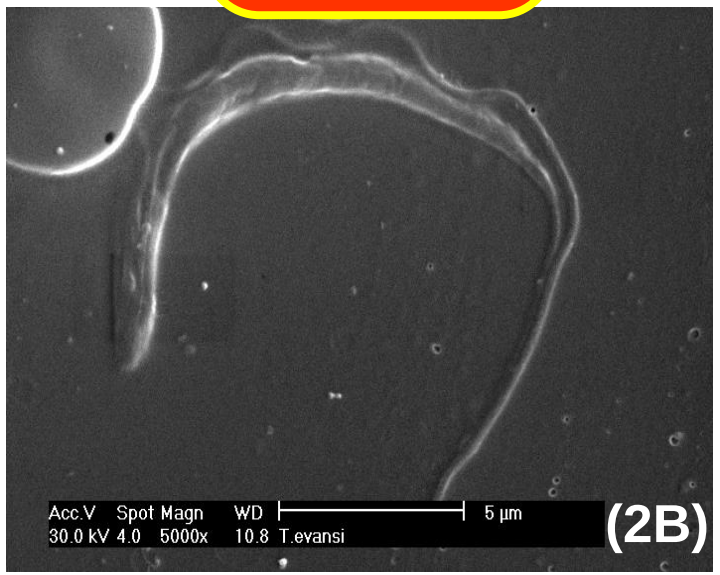
2nd hour

(2A)

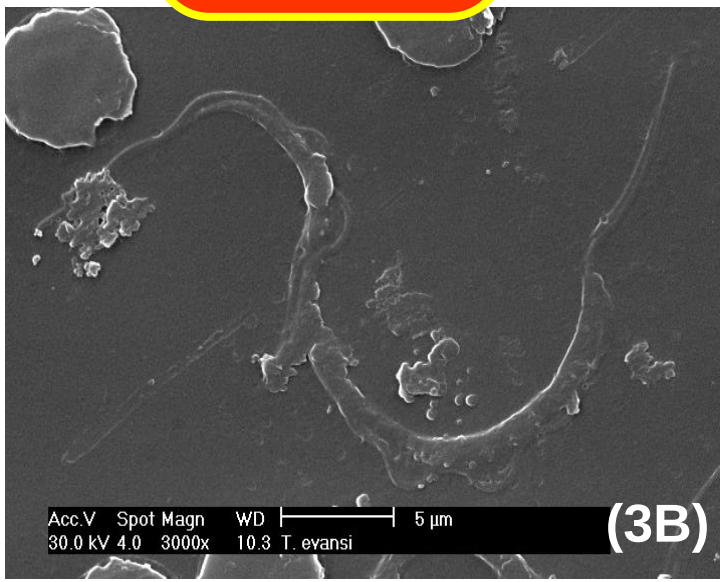


3rd hour

(3A)

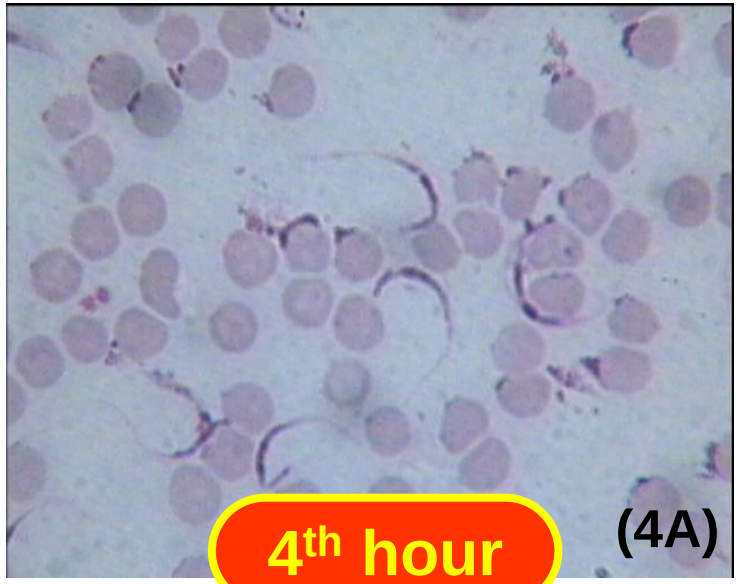


(2B)



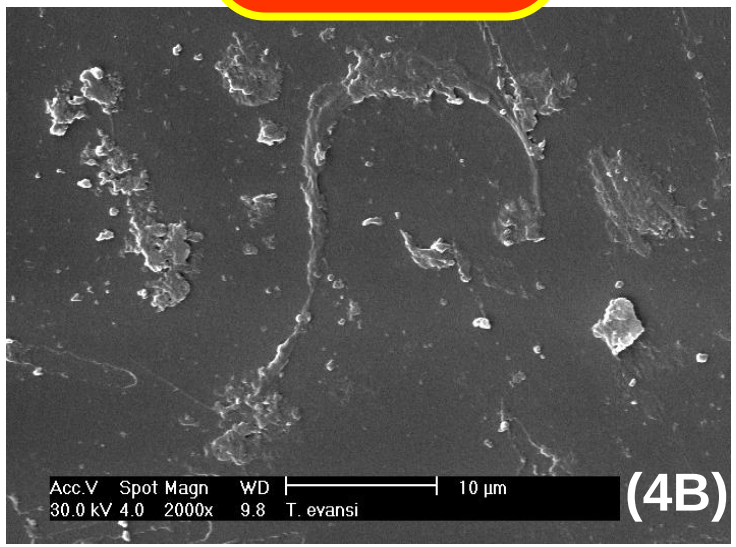
(3B)

Parasite Growth in Berenil-Treated Group (POS)

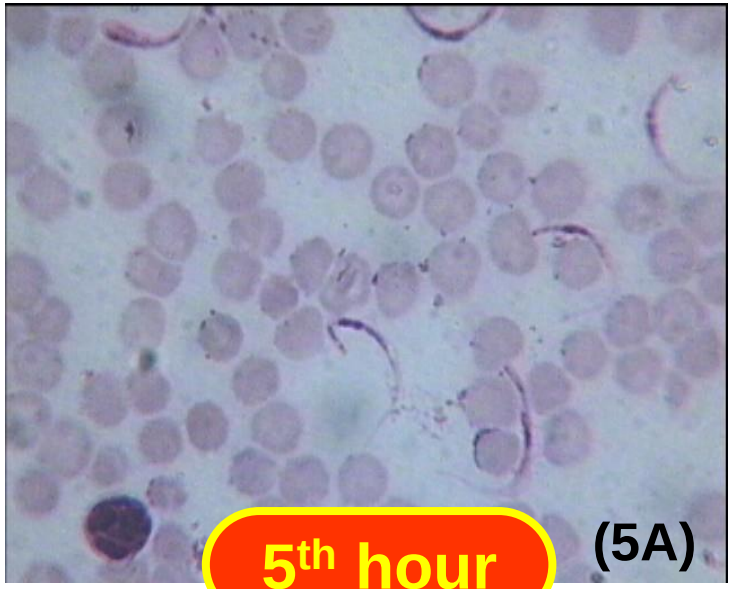


4th hour

(4A)

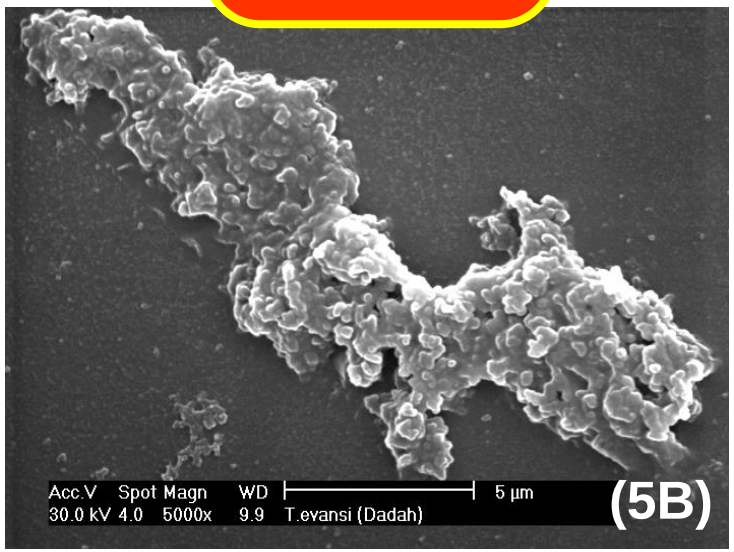


(4B)

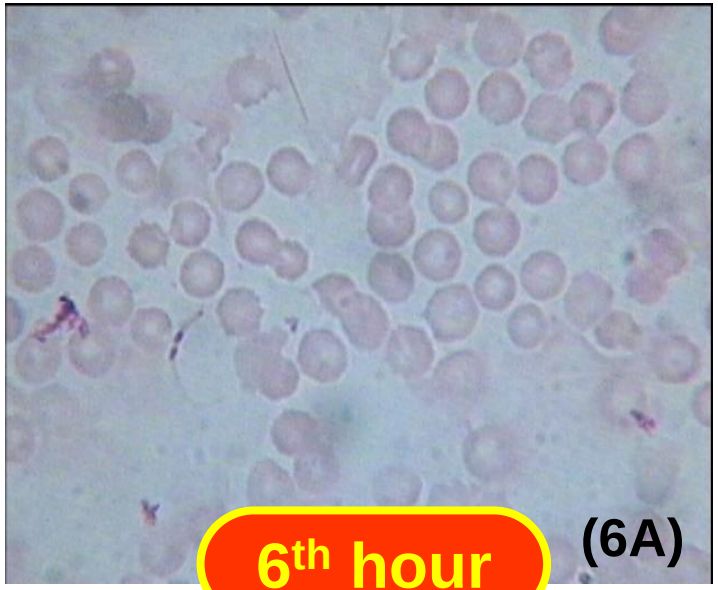


5th hour

(5A)

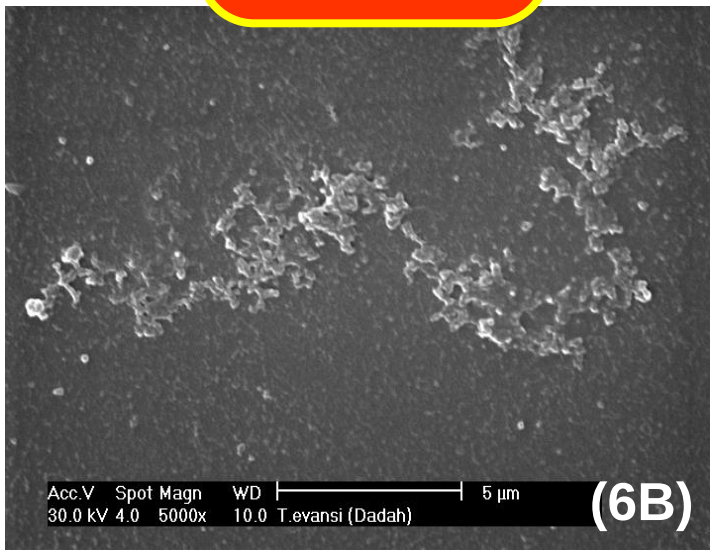


(5B)



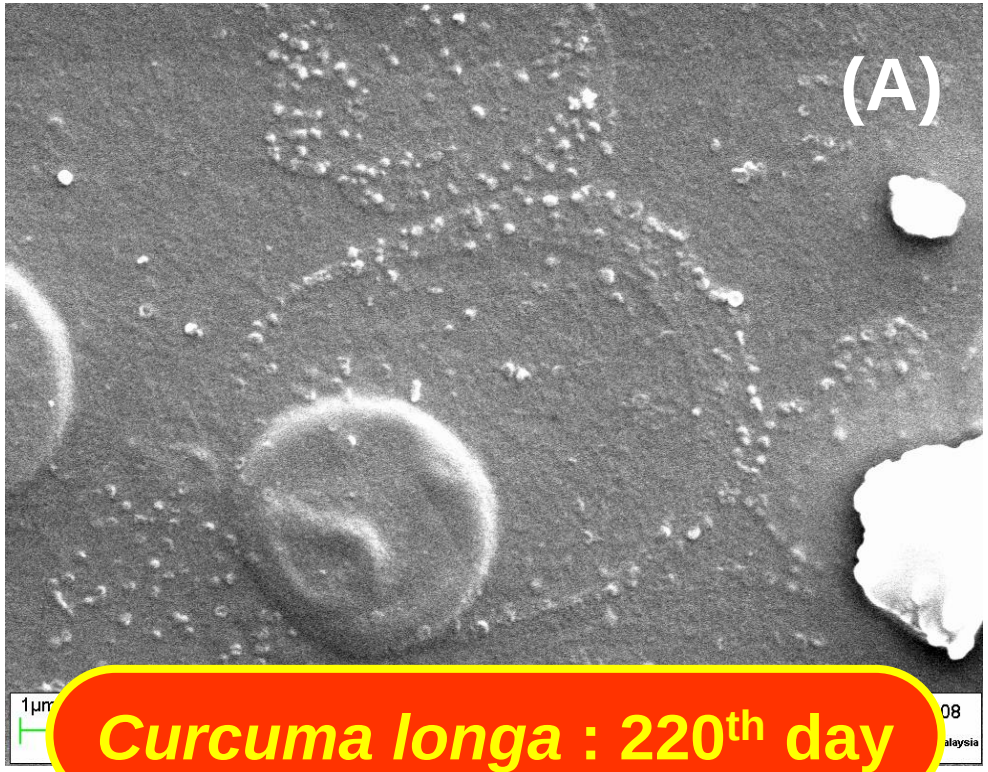
6th hour

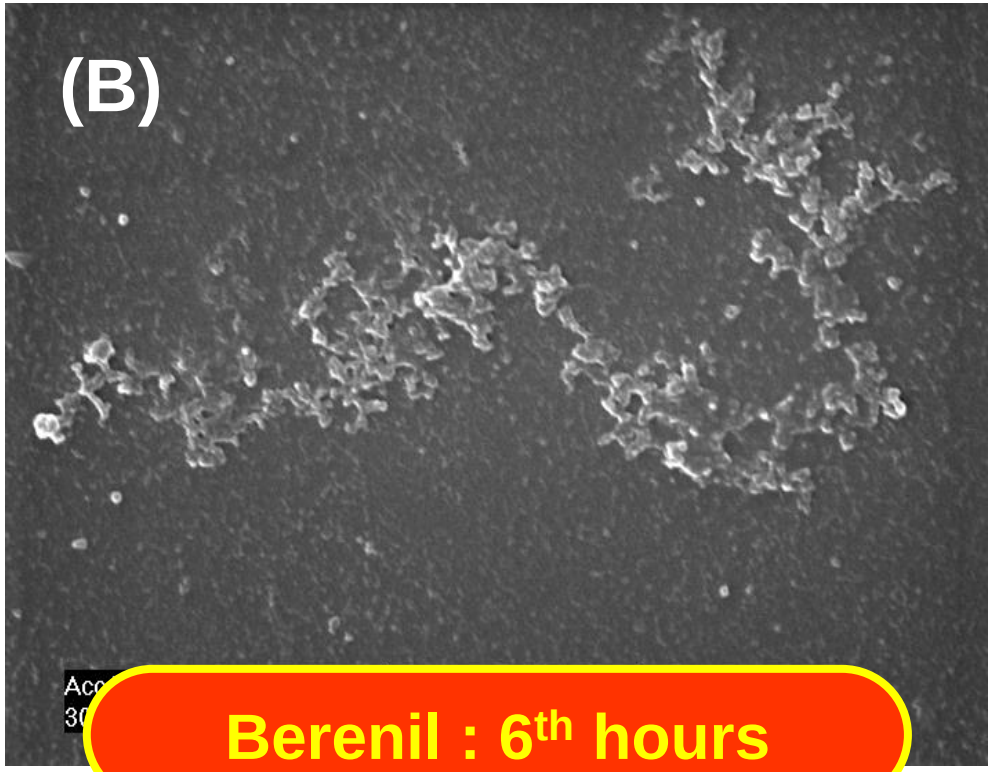
(6A)



(6B)

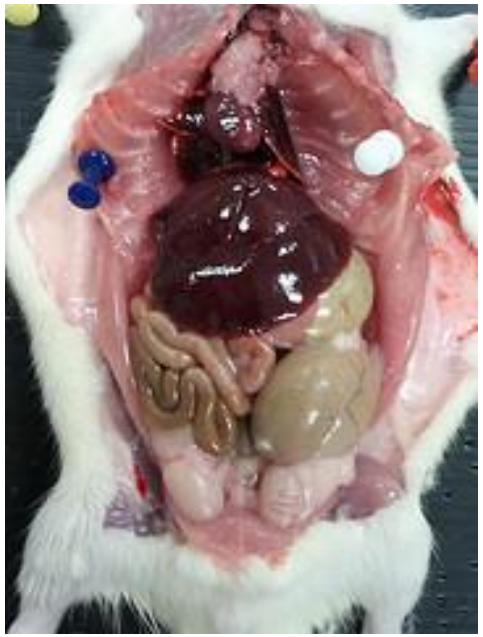
T. cucumerina VS Berenil





Scanning electron micrograph showed the morphological changes of *T. evansi* in PRE14 mice (0.2 mL 100 mg/kg bw *Curcuma longa* aqueous-extract) on 220th 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



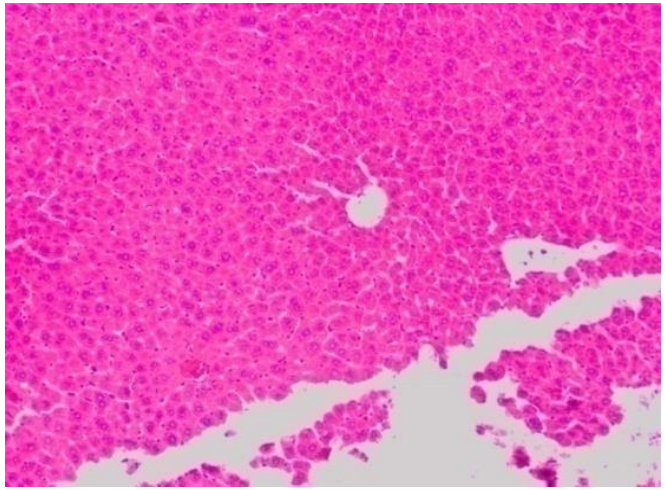
Test	TA	TB	TC	TD	CN	CI	NR	Unit
ALT (*)	41.81 ± 2.14	45.20 ± 1.13	67.57 ± 2.91	90.03 ± 2.02	41.03 ± 3.91	44.83 ± 1.11	40 – 93	IU/L
AST (*)	207.13 ± 2.04	208.02 ± 2.12	167.76 ± 2.27	187.01 ± 2.09	111.62 ± 1.19	134.43 ± 4.01	92 – 206	IU/L
ALP (*)	62.76 ± 2.33	59.4 ± 2.97	69.2 ± 2.90	68.03 ± 2.10	61.46 ± 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

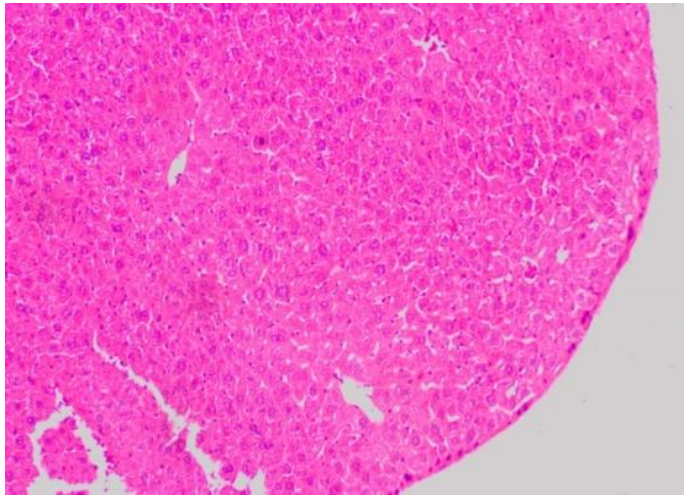
TA : Sub-acute regime – Daily treatment (28 days)
TB : Sub-acute regime – Daily treatment (28 days) 2 hours post-infection
TC : Sub-chronic regime – Daily treatment (90 days)
TD : Sub-chronic regime – Daily treatment (90 days) 2 hours post-infection
CN : Control regime – Normal mice without infection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

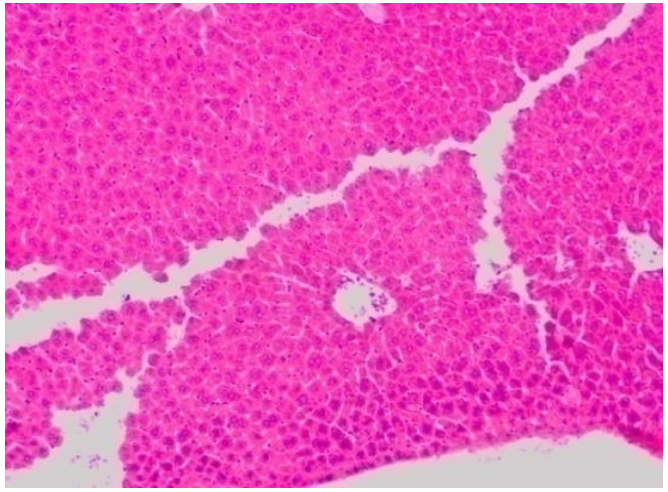
(*) ***All values were expressed as mean ± standard deviation (sd)***

Organ Histology For Toxicity Assessment

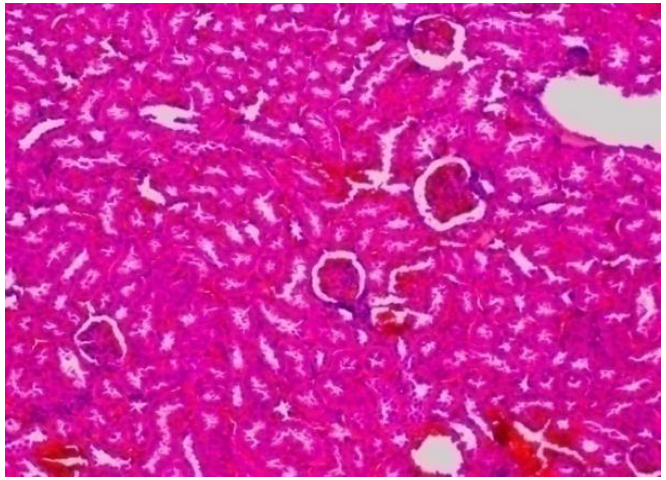
Liver

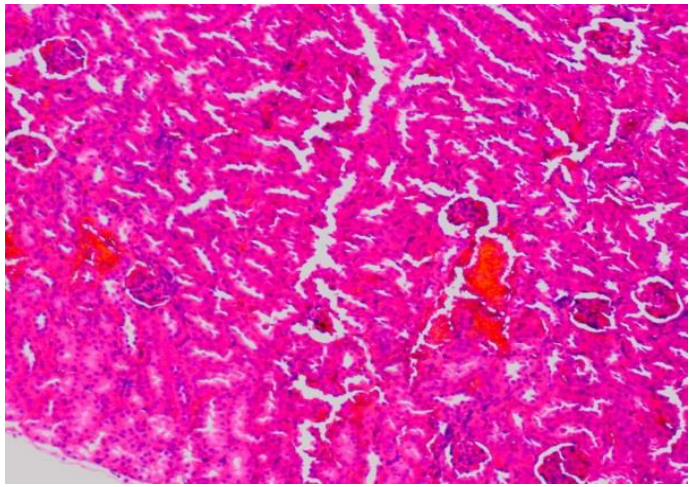


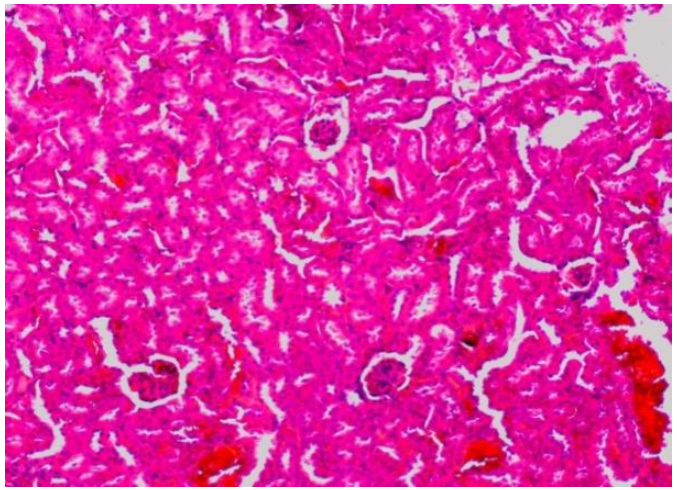




Kidney







Treatment (Acute)

Treatment (Sub-Acute)

Control

CONCLUSIONS

Hypothesis

- Longer prophylactic duration → longer survival period of the host.
- New wave of infection → mice is susceptible to infection (Kurup and Rajamohan 2011).
- Curative regime are less effective than other treatment regimens
- The action of β-turmerone ($C_{15}H_{22}O$) molecule in *Curcuma longa* against – thiol group of parasite enzymes in which crucial for parasite proliferation (Ma et al. 2012).
- Bioactive compound of isocurcumenol ($C_{15}H_{22}O_2$) in *Curcuma longa* inhibited the proteinase, thioredoxin reductase (Zhao et al. 2010)
- Compound α-curzerene ($C_{15}H_{20}O$) in *Curcuma longa* rhizomes interfered sugar absorption process in *Leishmania donovani* (Voon et al. 2014)

Absolute Hypothesis

EAT TURMERIC..!

NO HARM TO EAT AS MUCH AS YOU CAN

Absolute Hypothesis

Suggestions / Recommendations

Various solvents of *Curcuma longa* extract

Mechanism of action

In-vitro anti-trypanosomal screening

Concentration- & time-dependant alteration

Clinical & molecular approaches

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

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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

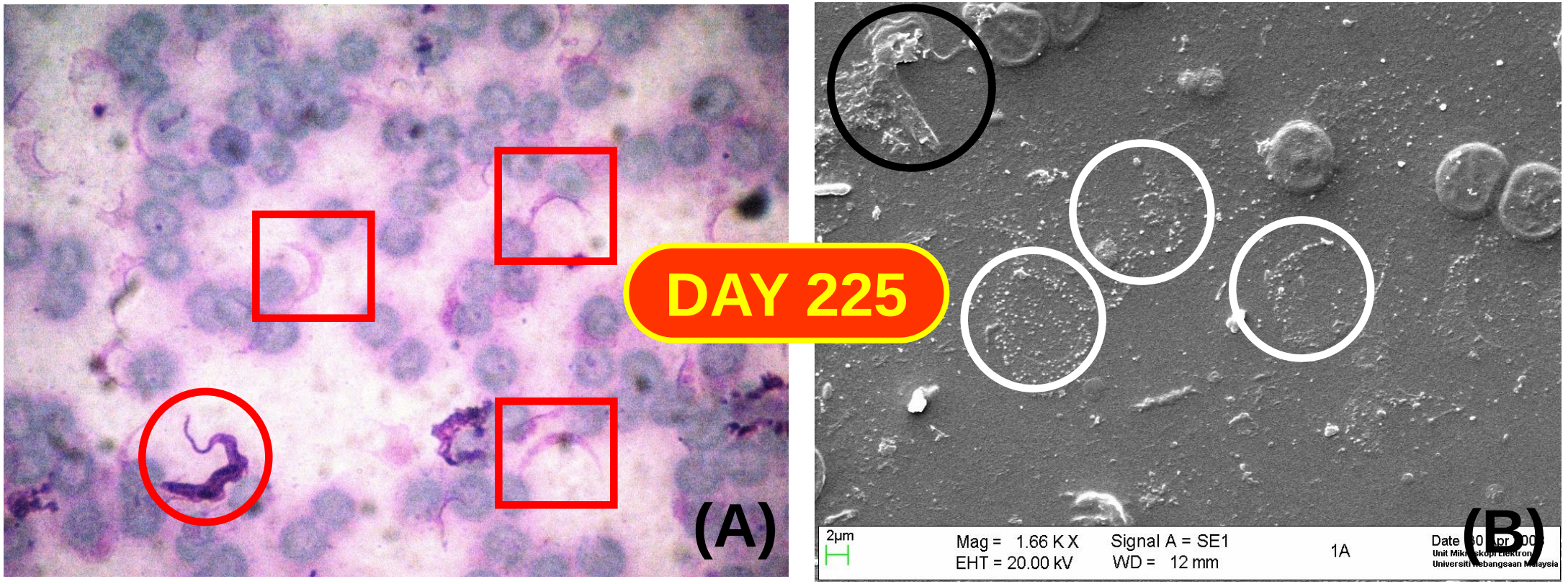
Economic Growth & Biotechnology Sector

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

Current Issues of *T. evansi*

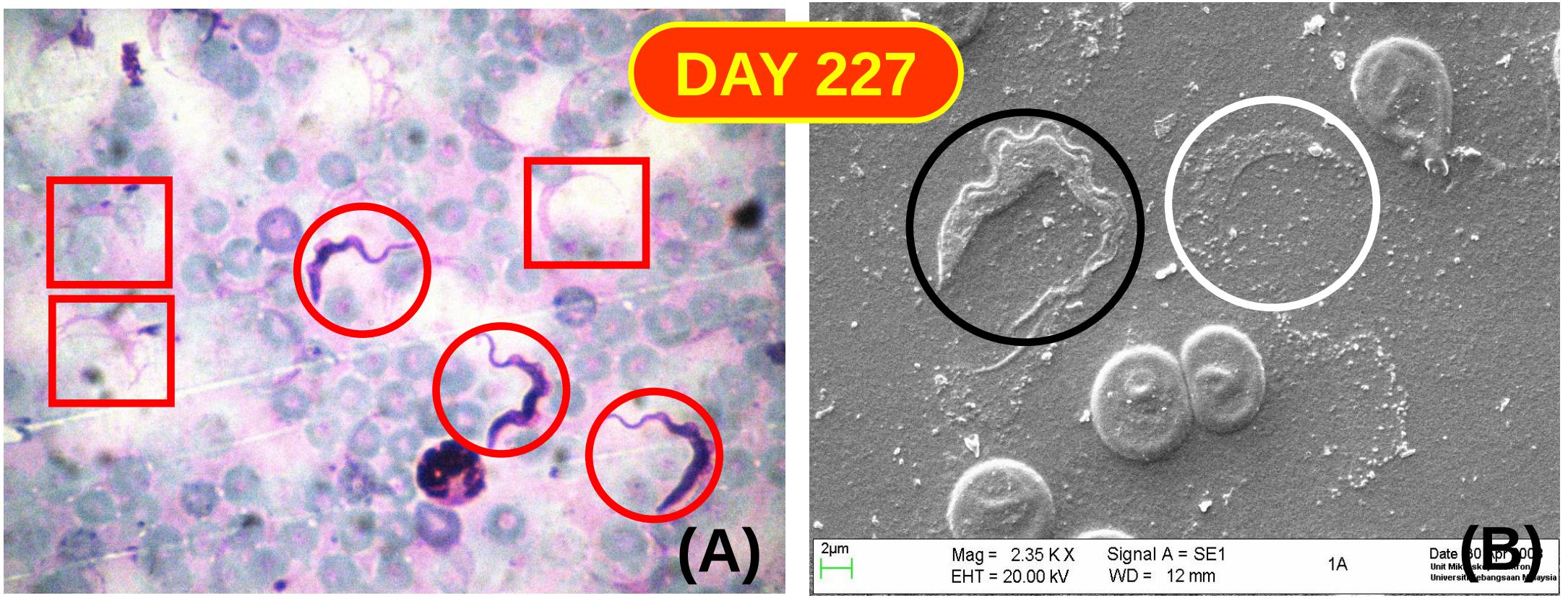
- Trans-host boundary : animal → human (Assam India 2008)

Parasite Survival In PRE14 Mice Group : 225th Day



Reemerged of *T. evansi* which survived in PRE14 group mice on day 225 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 227 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 228

‘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