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South East Asia One Health University Network

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INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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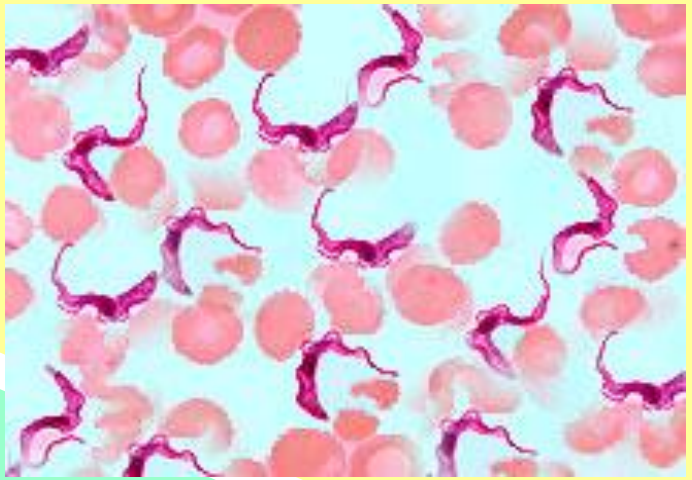
One Health (OH) Concept on the Assessment of *In-Vivo* Antiparasitic Activity of Nerolidol Against the Growth and Survival of Zoonotic Haemoflagellate Protozoa, *Trypanosoma evansi*



Assistant Prof. Dr. Mohd Shukri Bin Baba

International Islamic University Malaysia

INTRODUCTION

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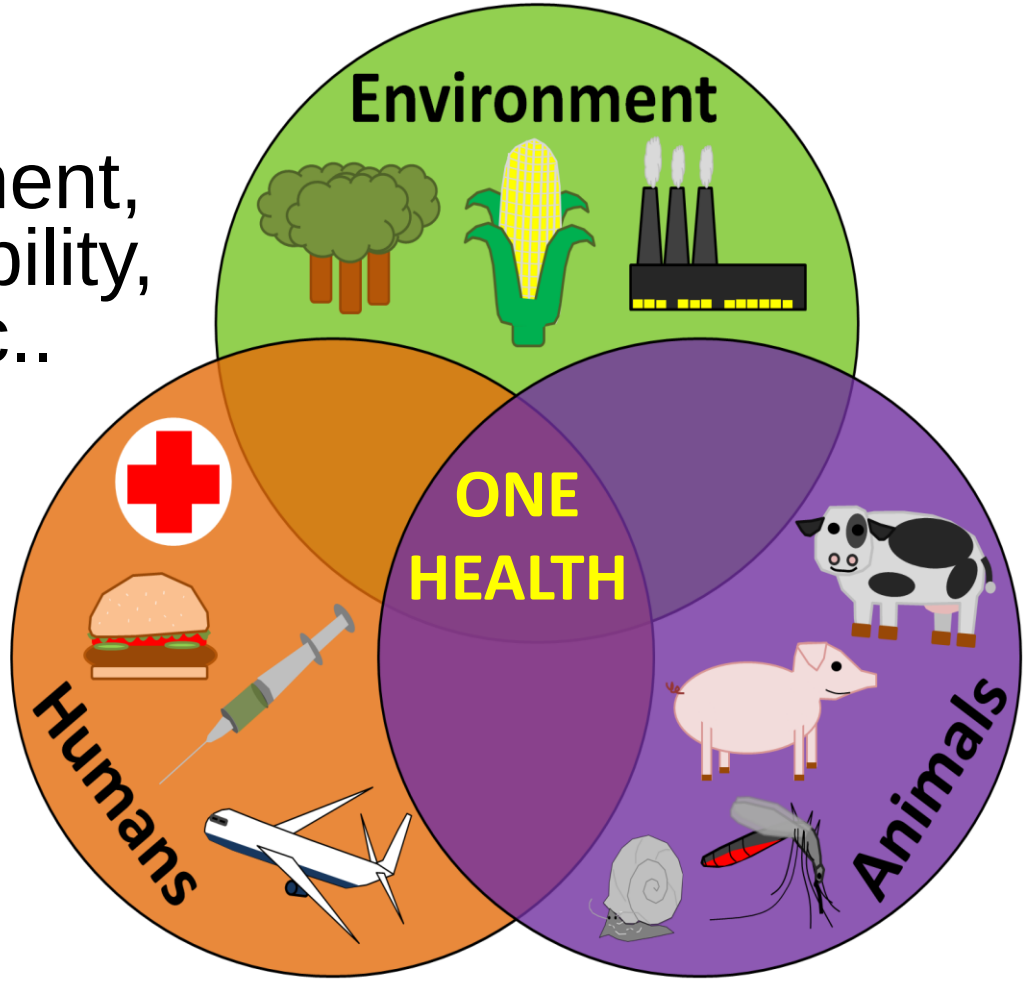
INTRODUCTION

INTRODUCTION

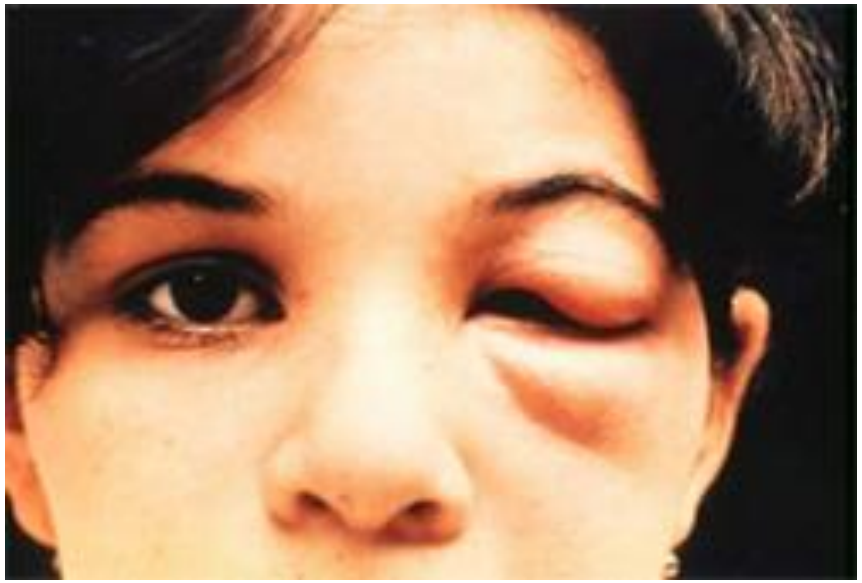
One Health (OH) Concept

- Recognizes interrelationship between animal, human and environmental health, locally and globally.
- Holistically embraces a very broad scope: biodiversity, rural development, food security, ecosystem sustainability, policy issues, wildlife diseases, etc..
- Initiate worldwide strategies for interdisciplinary collaboration & communication in all aspect of healthcare and services
- Everyone involved in OH could identify their specific role within it



INTRODUCTION

Trypanosomiasis



- Vector borne → haemoflagellate & unicellular protozoa
  - *T. b. gambiense* → chronic HAT
  - *T. b. rhodesiense* → acute HAT
  - *T. cruzi* → Chagas disease
- Atypical human trypanosomiasis:
  - *T. vivax* → nagana (cattle & wild)
  - *T. lewisi* → *Rattus* & other rodents
  - *T. congolense* → nagana (camels, dogs, horses, pigs, ruminants)
  - *T. evansi* → surra (mammals)

INTRODUCTION

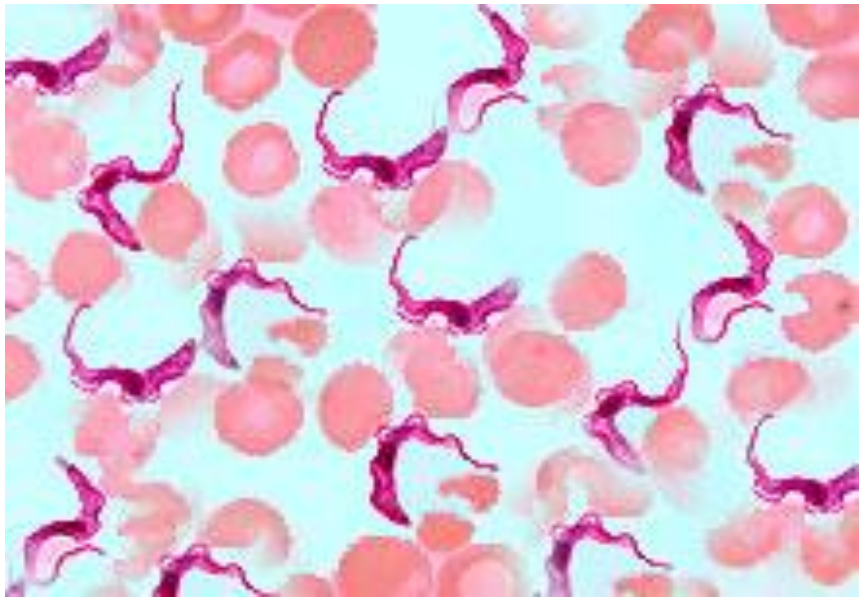
Atypical Human Trypanosomiasis (aHT) (Philippe et al. 2013)

Patient Number	Location	Trypanosome Species/Sub-species	Date	Parasite Identification Method[a]	Fever	Treatment	Outcome
1	Ghana	*T. vivax*	1917	Morphology	ND	ND	ND
2	Pasteur Institute	*T. b. brucei*	1930[b]	Morphology	ND	ND	ND
3	Congo	*T. b. brucei*	1947[c]	Morphology	Present	None	Self-cure
4	Ethiopia	*T. b. brucei*	1987	Morphology BIIT	ND	ND	Cure
5	Ghana	*T. b. brucei*	2003	PCR	Present	None	Self-cure
6	Côte d'Ivoire	*T. congolense*	1998	PCR	Present	Pentamidine	Cure
7	India	*T. evansi*	1977[b]	Morphology	Present	Atoxyl	Cure
8	Sri Lanka	*T. evansi*	1999	Morphology	Present	None	Self-Cure
9	India, Seoni	*T. evansi*	2004	PCR	Present	Suramin	Cure
10	India, Kolkata	*T. evansi*	2005	Morphology	Present	None	Death
11	Egypt	*T. evansi*	2010	Morphology	Present	ND	Cure
12	Malaysia	*T. lewisi*	1933	Morphology	Present	None	Self-cure
13	India, Parsda	*T. lewisi*	1974	Morphology	Present	None	Self-cure
14	India, Parsda	*T. lewisi*	1974	Morphology	Present	None	Self-cure
15	The Gambia	*T. lewisi*-like	2003	PCR/S	Present	Melarsoprol	Cure
16	Thailand	*T. lewisi*-like	2003	PCR/S	Present	Antibiotic	Cure
17	India, Mumbai	*T. lewisi*	2006	Morphology	Present	None	Self-cure
18	India, Pune	*T. lewisi*	2007	PCR	Present	Suramine	Death
19	India, Bagpat	*T. lewisi*	2010	PCR/S	Present	Pentamidine	Cure

INTRODUCTION

Trypanosoma evansi

- Animal haemoflagellated protozoa → evolutionarily from *T. brucei*
- 1st discovered in 1880 by Sir Griffith Evans in Punjab, India
- Malaysia in 1903 → cow & sheep migration from Thailand
- Atypical human trypanosomiasis (aHT) → 5 human zoonotic cases in India, Egypt and Sri Lanka
- Bad impact & challenges on both human and veterinary medicine



Vectors of Trypanosomiasis

INTRODUCTION

Vectors of Atypical Human Trypanosomiasis (aHT)

Argasidae tick / *Ornithordorus*

Horsefly / Tabanidae

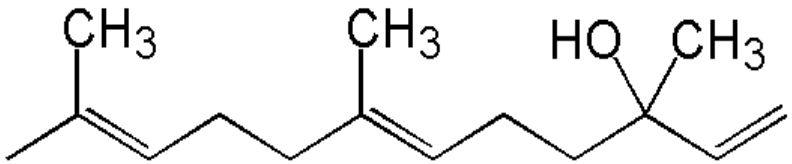
Muscidae fly / *Stomoxys*

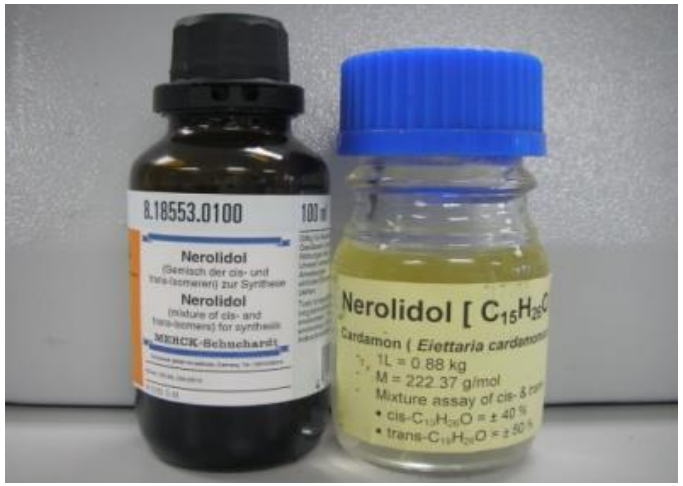
Horn fly / *Haematobia*

INTRODUCTION

Elettaria cardamomum & Nerolidol

- "*Queen of Spice*" (Ravindran 2010)
- South Asia, SEA, Middle East, Africa and Europe.
- In 100gm → 300kcal, 68g CHO, 15g protein, 28g fibers & no cholesterol
- Vitamin A & C, Na, K, Ca, Fe, Mn, P, Cu, Mg & Zn (Cox et al. 2000)
- Nerolidol ($C_{12}H_{26}O$) → therapeutic effects & significant biological activities (Gao et al. 2008)





INTRODUCTION

Nerolidol : Testimonies

- In-vitro inhibits 95% of *Leishmania amazonensis* and *L. braziliensis* promatigotes growth (Denise at el. 2010)
- Inhibits the synthesis of peptidoglycan molecules in bilayer lipid structure of organism's plasma membrane (Ogunlana et al. 2013)
- Anti-colorectal tumour activity with 68% inhibition rate towards human colon adenocarcinoma cells HCA-2 and HCA-7 (Gayathri et al. 2010)
- Curing asthma and bronchitis symptoms by increasing blood circulation to the lungs (Berhe et al. 2009)
- Oil-based of nerolidol exhibits anti-gonorrhea and anti-nephritis property in male rabbit's urethra (Turi et al. 2011)

INTRODUCTION

Rationale of the Study

Reliability of Berenil

- Unaffordable → expensive in certain regions
- Wrong dosage & concentration → side effects

Economic Growth & Biotechnology Sector

- Natural products → newest main focus for developing nations
- Widely unutilized lands → profitable with cardamom plants
- Cardamom → consumable & easily manipulated herbs
- Surra disease → influenced productivity of the human & livestock

Current Issues of *T. evansi*

- Trans-host boundary: animal → human (Assam India, 2008)
- Strain from India, Egypt & Indonesia are now MDR-strain
- Distribution of vectors → potentially could be trans-continents

MATERIALS & METHODS

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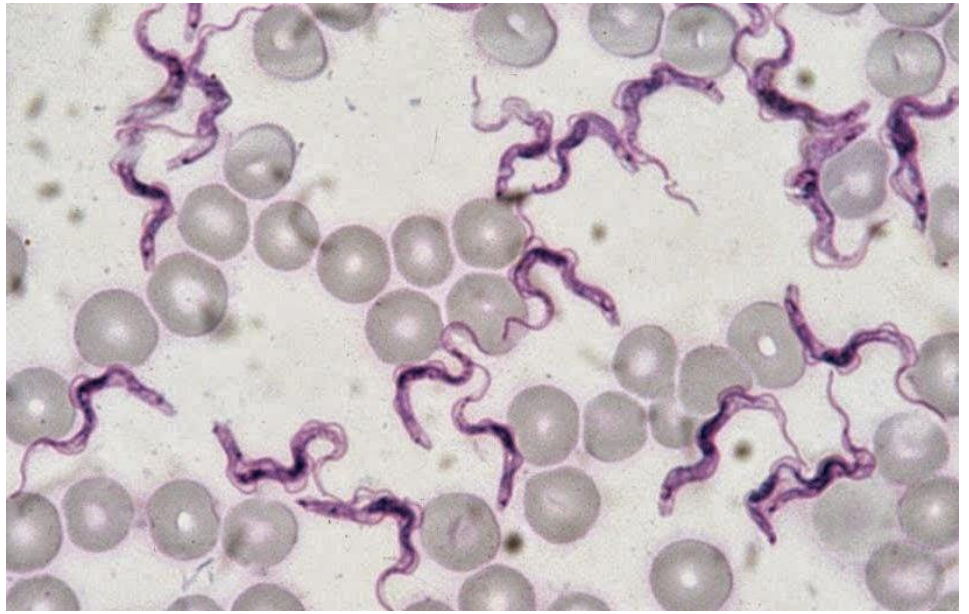
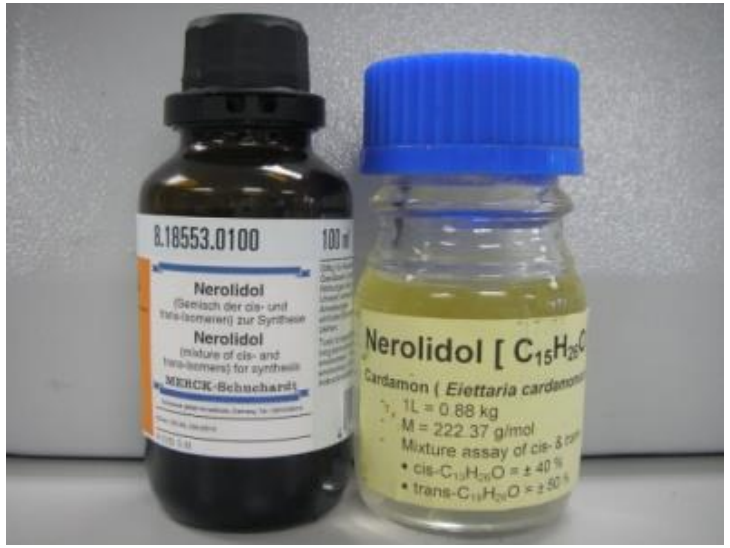
MATÉRIAUX & MÉTHODES

MATERIALS

Experimental Items

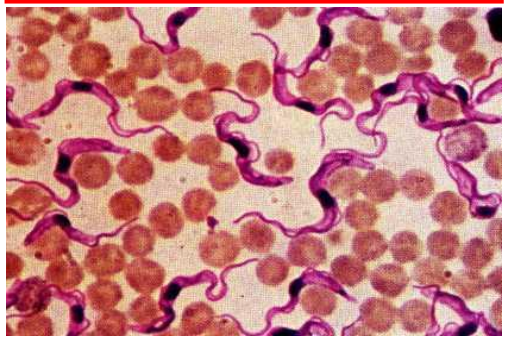




METHODOLOGY

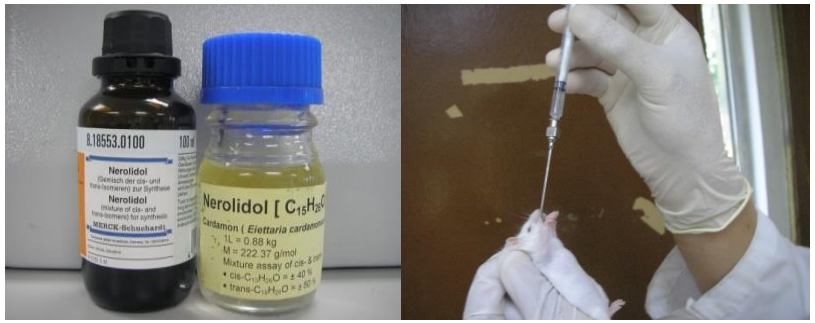
Work Flow

T. evansi stock inoculation



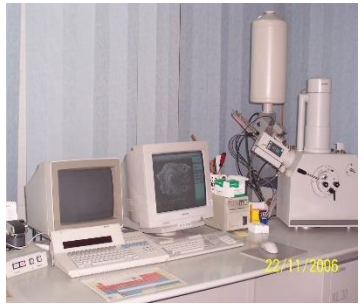
T. evansi i.p. administered (5 × 10^3 *T. evansi* / mice)

0.1 mL 15 µL/mL nerolidol- dH_2O solution: orally administered



Giemsa blood smear 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

METHODOLOGY

Experimental Design

GROUP	REGIME	CODE : DESCRIPTION	DOSAGE
TREATMENT	PREVENTIVE	**PRE14** : 14 days pre-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
		PRE7 : 7 days pre-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
		PRE3 : 3 days pre-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
	CONCURRENT	**CON** : 30 minutes post-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
	CURATIVE	**CUR3** : 3 days post-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
		CUR7 : 7 days post-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
		CUR14 : 14 days post-infection	0.1 mL 10 µg/mL nerolidol-dH_2O
CONTROL	POSITIVE	**POS** : Berenil (Sigma-Aldrich)	0.01 mL 3.5 mg/kg bw
	NEGATIVE	**NEG** :0.9 % Normal Saline	0.1 mL 0.9% normal saline
	LETHAL	**LWT** : Infection without treatment	5×10^3 *T. evansi* / mice (I.P.)

Mice : ICR / ♂ / 6 – 8 weeks old / 25 – 30 g bw / n = 6 per group
Nerolidol and normal saline → daily administered (oral) until the mice die.
Berenil → administered intraperitoneally (I.P.) as single dose once the parasitemia density = 20 - 30 %.
Nerolidol (Sigma-Aldrich, Malaysia) → extracted from *Eiettaria cardamomum* (cardamom).

RESULTS & DISCUSSIONS

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RÉSULTATS & DISCUSSIONS

RESULTS & DISCUSSION

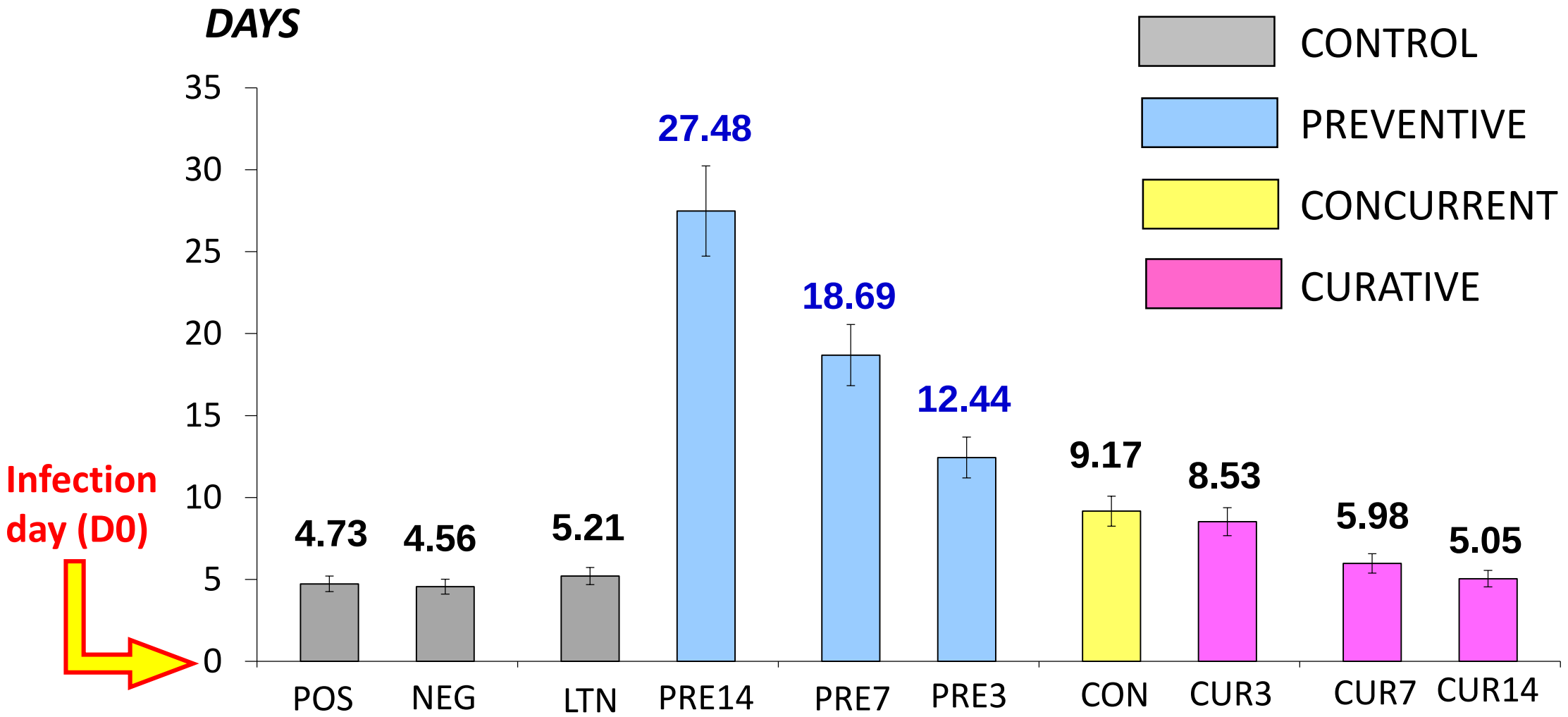
Parasite Pre-Patent Period vs Mice Survival Time

GROUP	REGIME	GROUP CODE	PARASITE PRE-PATENT PERIOD	MICE SURVIVAL TIME
TREATMENT	PREVENTIVE	PRE14	**27.48 ± 1.5**	**282.58 ± 6.6**
		PRE7	18.69 ± 2.2	112.48 ± 2.9
		PRE3	12.44 ± 0.2	55.49 ± 1.0
	CONCURRENT	CON	9.17 ± 3.1	21.60 ± 5.3
	CURATIVE	CUR3	8.53 ± 2.7	18.45 ± 4.0
		CUR7	5.98 ± 0.5	16.94 ± 1.1
		CUR14	5.05 ± 3.9	15.47 ± 3.7
CONTROL	POSITIVE	POS	4.73 ± 1.1	**> 365**
	NEGATIVE	NEG	4.56 ± 0.3	15.01 ± 4.8
	LETHAL INFECTION	LTN	5.21 ± 0.7	16.71 ± 2.1

Berenil (POS) was administered intraperitoneally (I.P.) as single dose once the parasitemia density reached 28.17 % on day 9 post-infection.

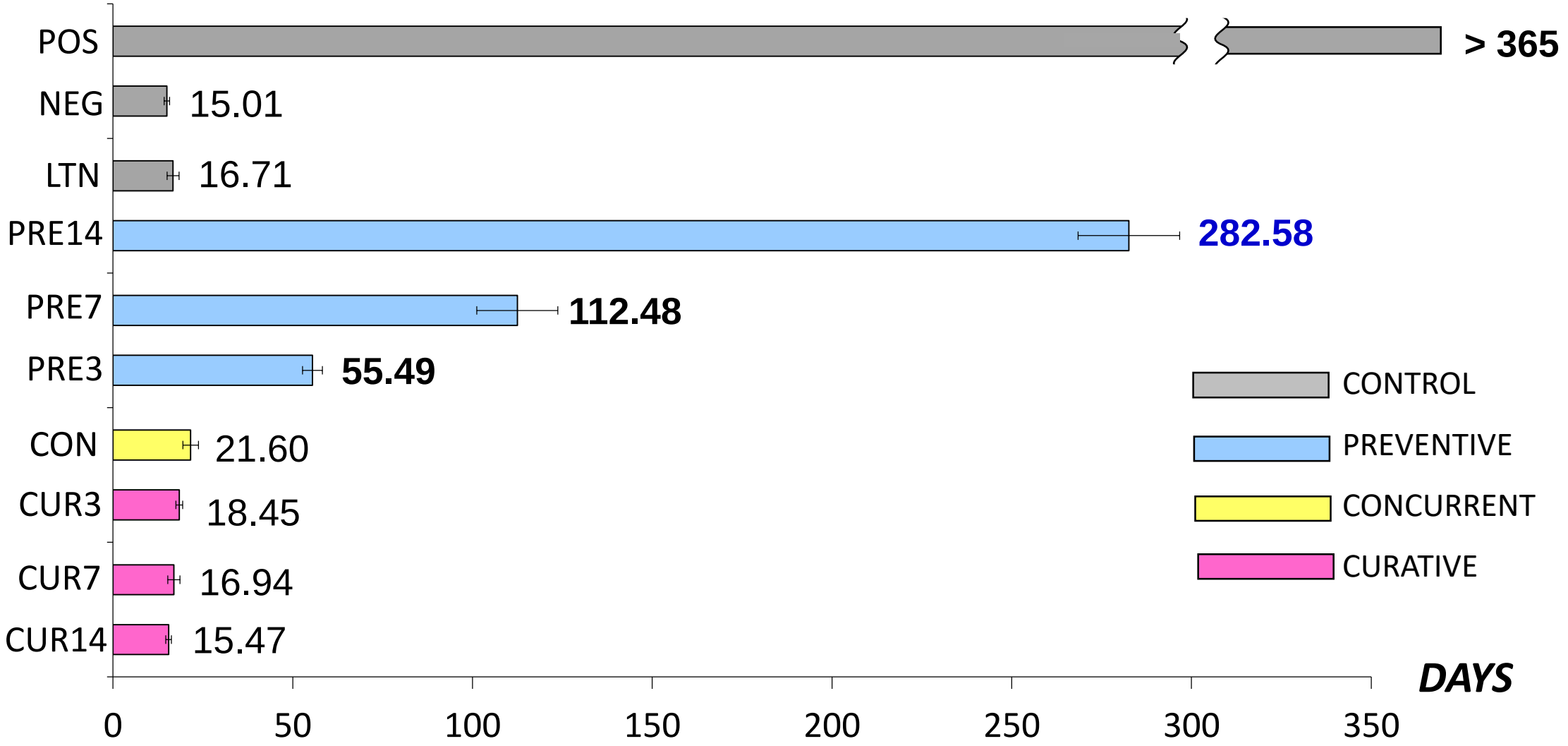
RESULTS & DISCUSSION

Parasite Pre-Patent Period



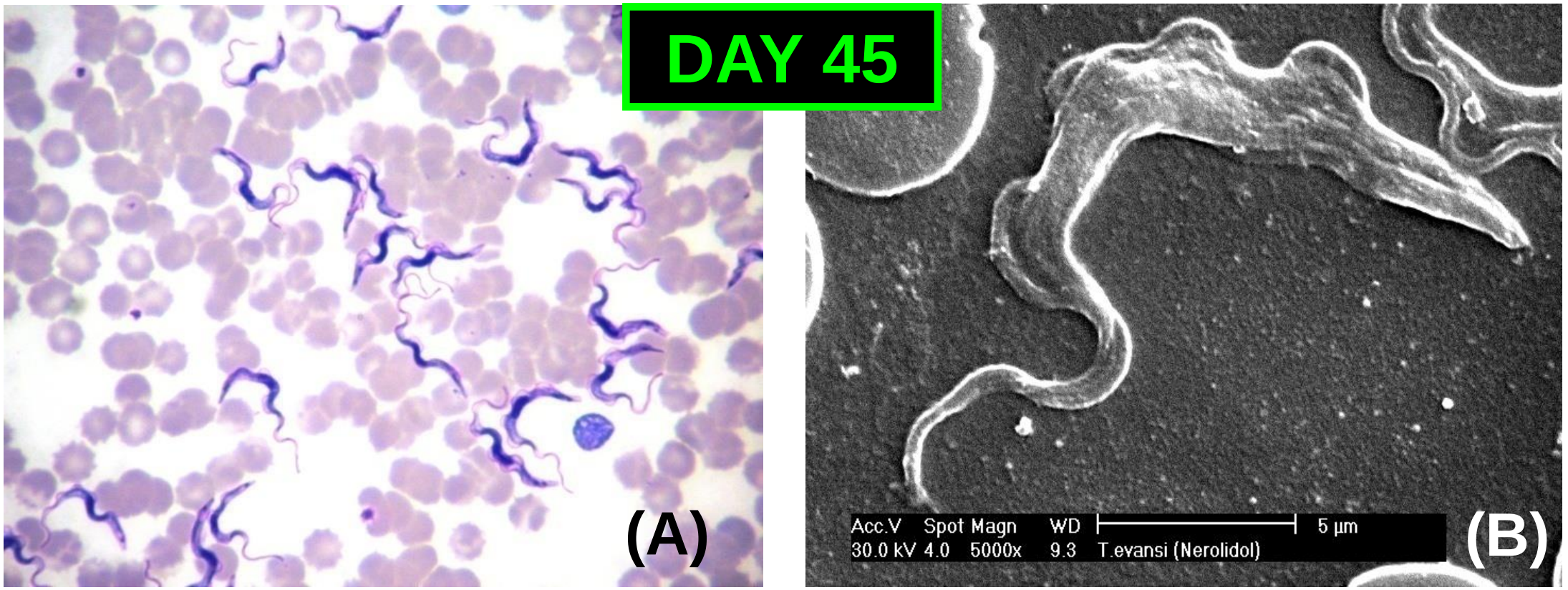
RESULTS & DISCUSSION

Mice Survival Time



RESULTS & DISCUSSION

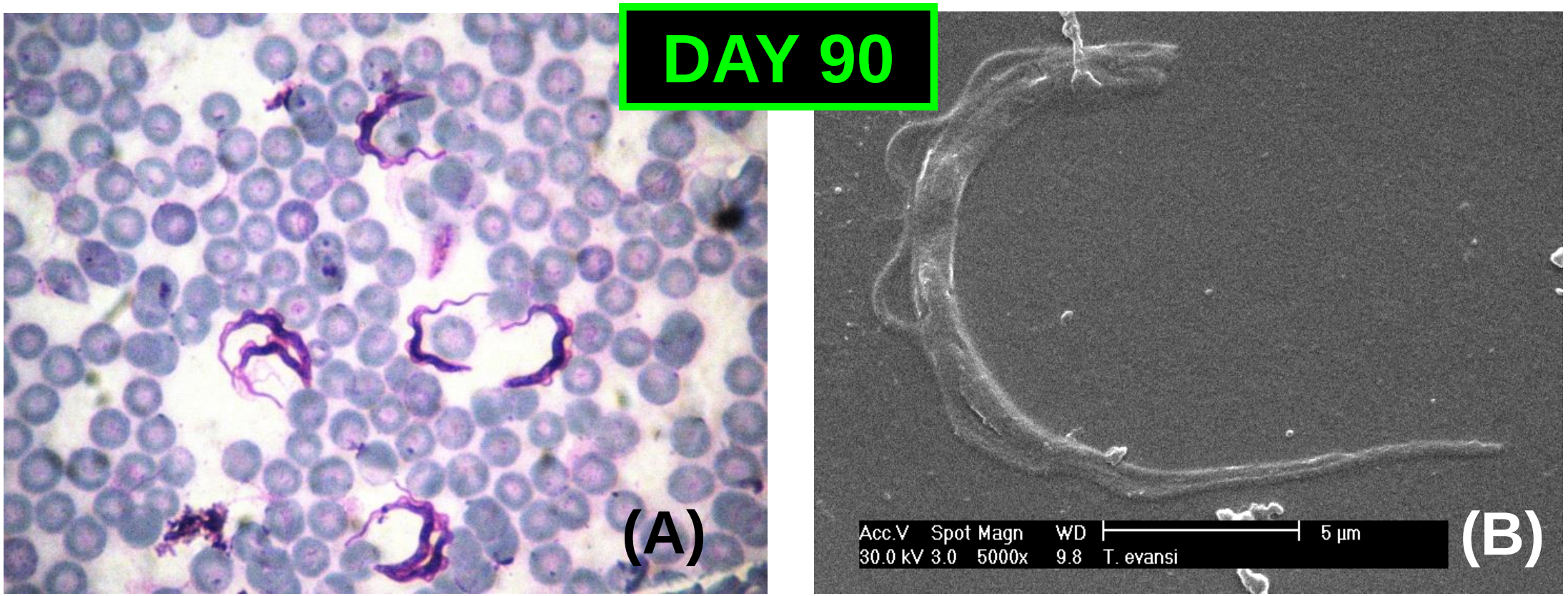
Parasite Growth & Survival in PRE14 Mice : Day 45



Giemsa thin blood smear of PRE14 mice group taken on day 45 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

RESULTS & DISCUSSION

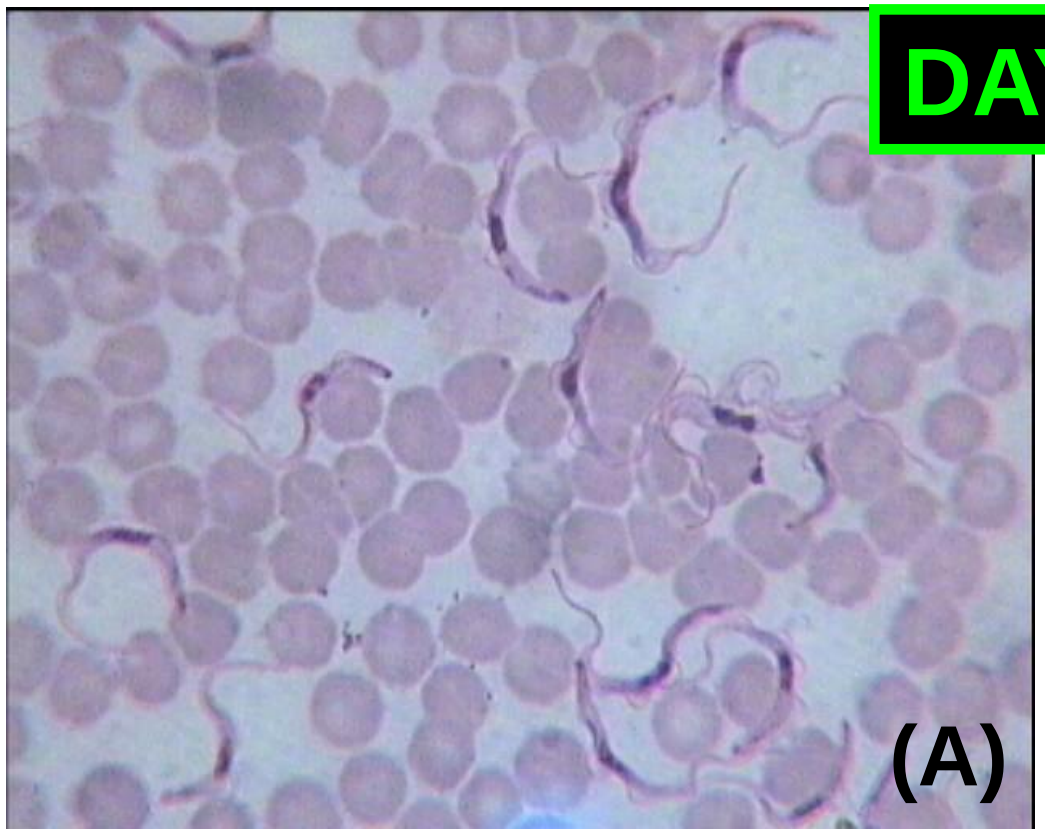
Parasite Growth & Survival in PRE14 Mice : Day 90

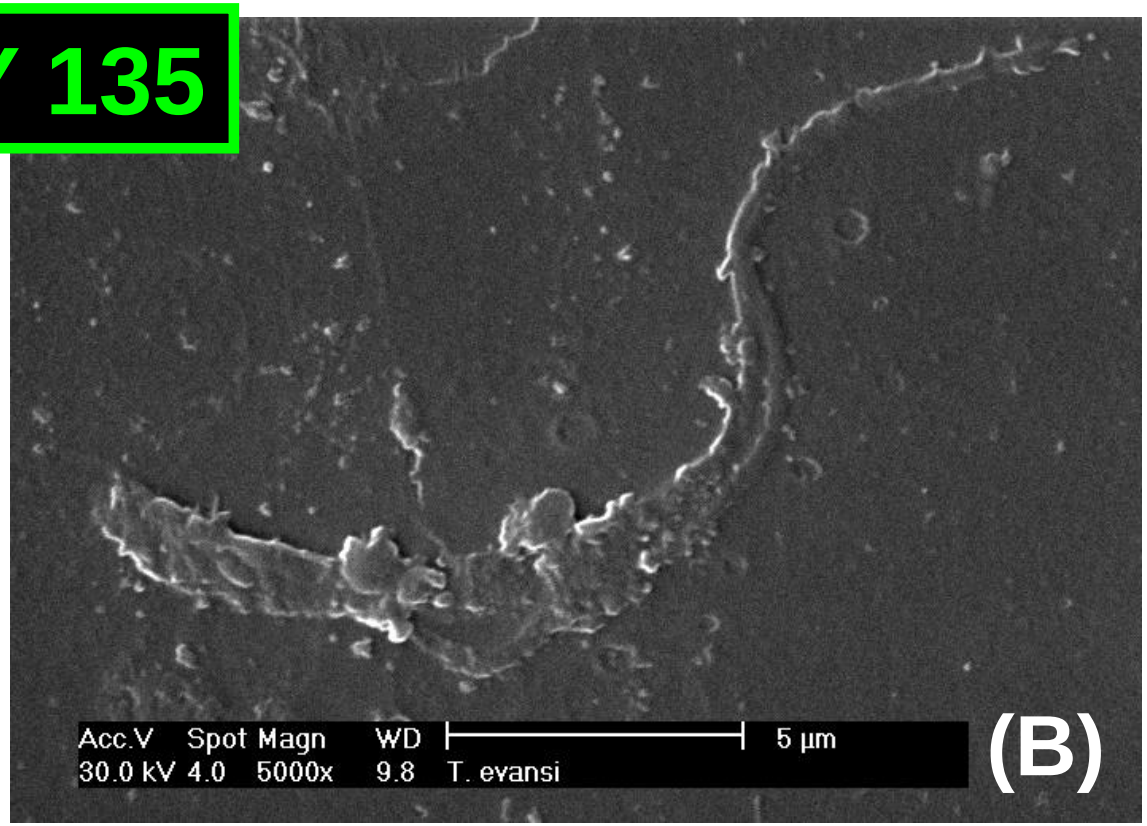


Giemsa thin blood smear of PRE14 mice group taken on day 90 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

RESULTS & DISCUSSION

Parasite Growth & Survival in PRE14 Mice : Day 135

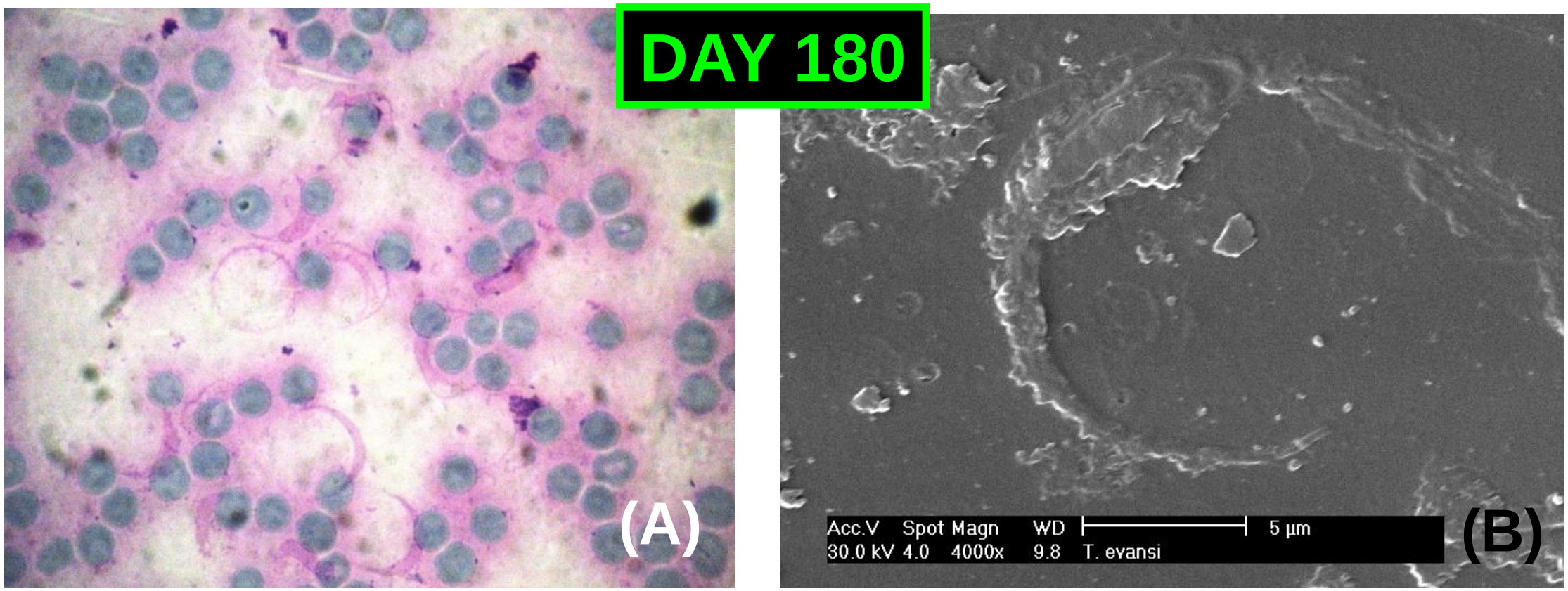




Giemsa thin blood smear of PRE14 mice group taken on day 135 as observed under x100 magnification of light microscope (A) and x5000 magnification of SEM (Phillips XL30, UK) (B)

RESULTS & DISCUSSION

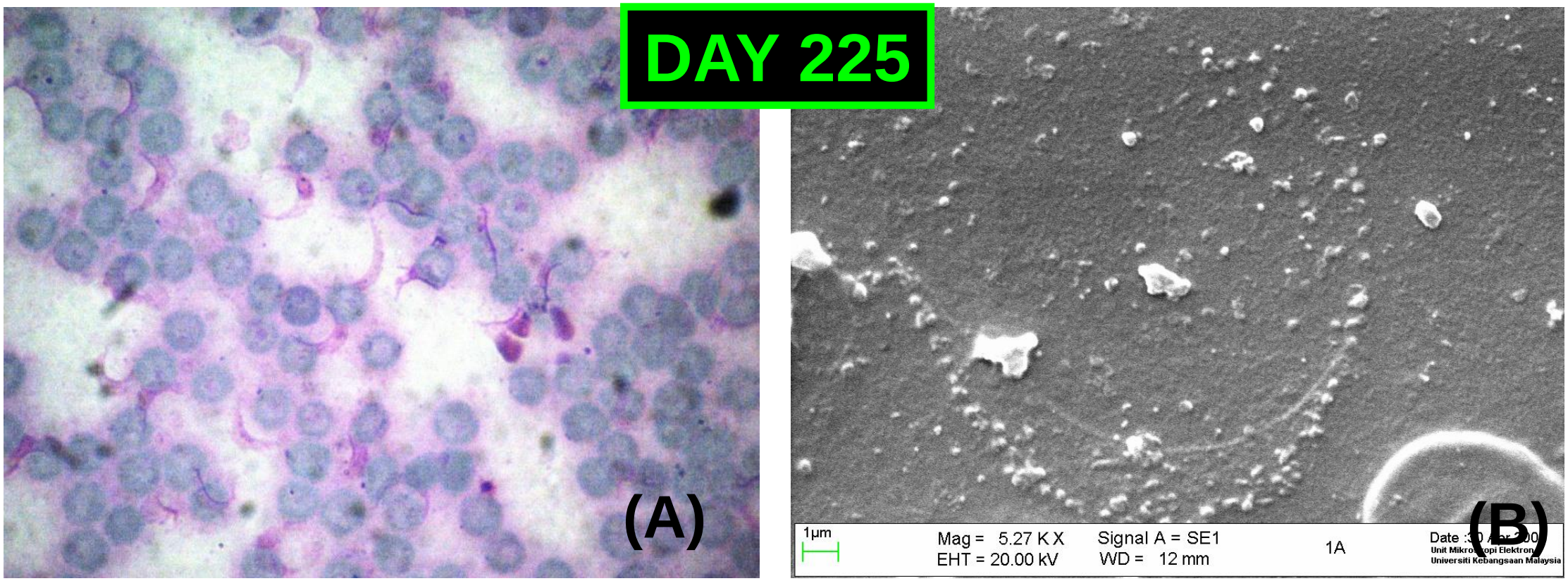
Parasite Growth & Survival in PRE14 Mice : Day 180



Giemsa thin blood smear of PRE14 mice group taken on day 180 as observed under x100 magnification of light microscope (A) and x4000 magnification of SEM (Phillips XL30, UK) (B)

RESULTS & DISCUSSION

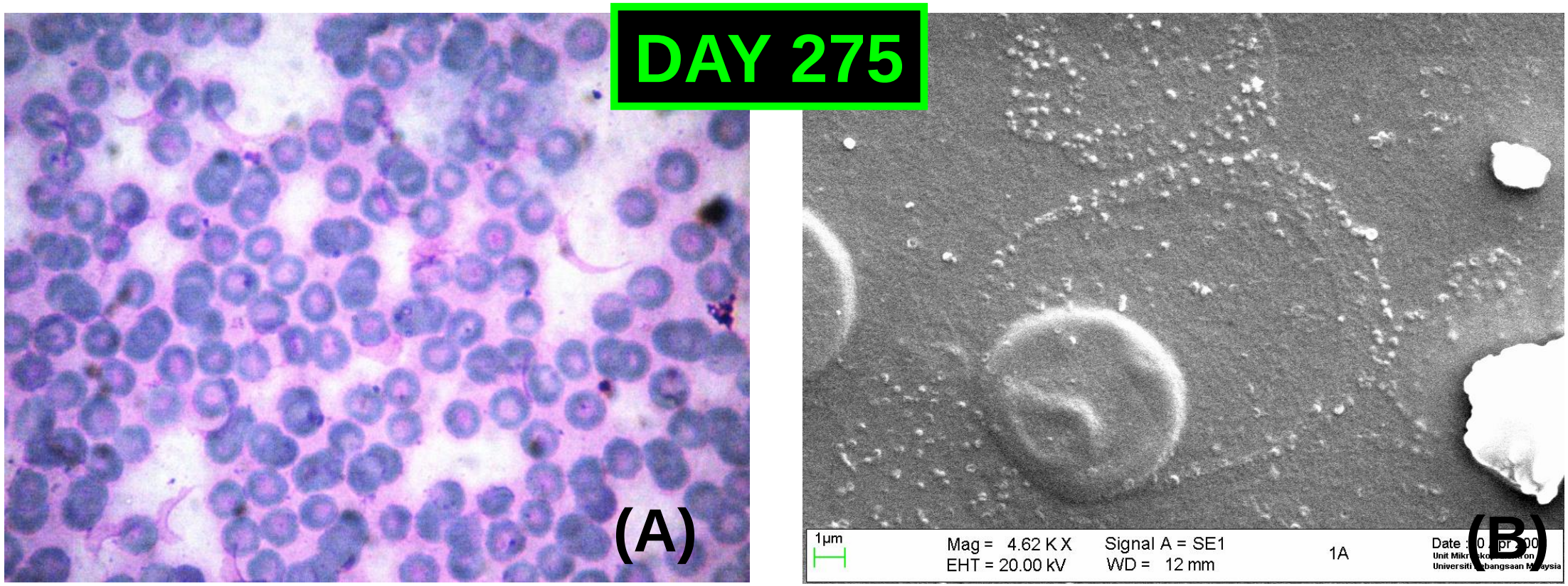
Parasite Growth & Survival in PRE14 Mice : Day 225



Giemsa thin blood smear of PRE14 mice group taken on day 225 as observed under x100 magnification of light microscope (A) and x5200 magnification of SEM (Leo 1450VP, Japan) (B)

RESULTS & DISCUSSION

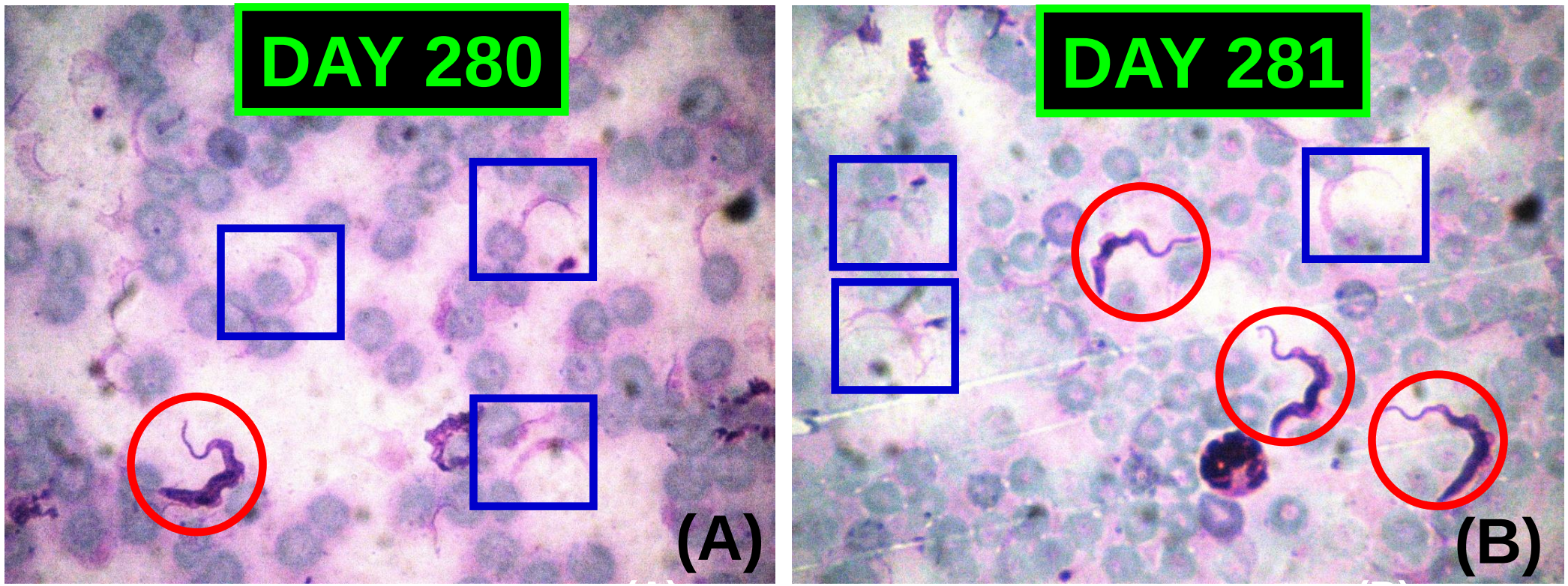
Parasite Growth & Survival in PRE14 Mice : Day 275



Giemsa thin blood smear of PRE14 mice group taken on day 275 as observed under x100 magnification of light microscope (A) and x4600 magnification of SEM (Leo 1450VP, Japan) (B)

RESULTS & DISCUSSION

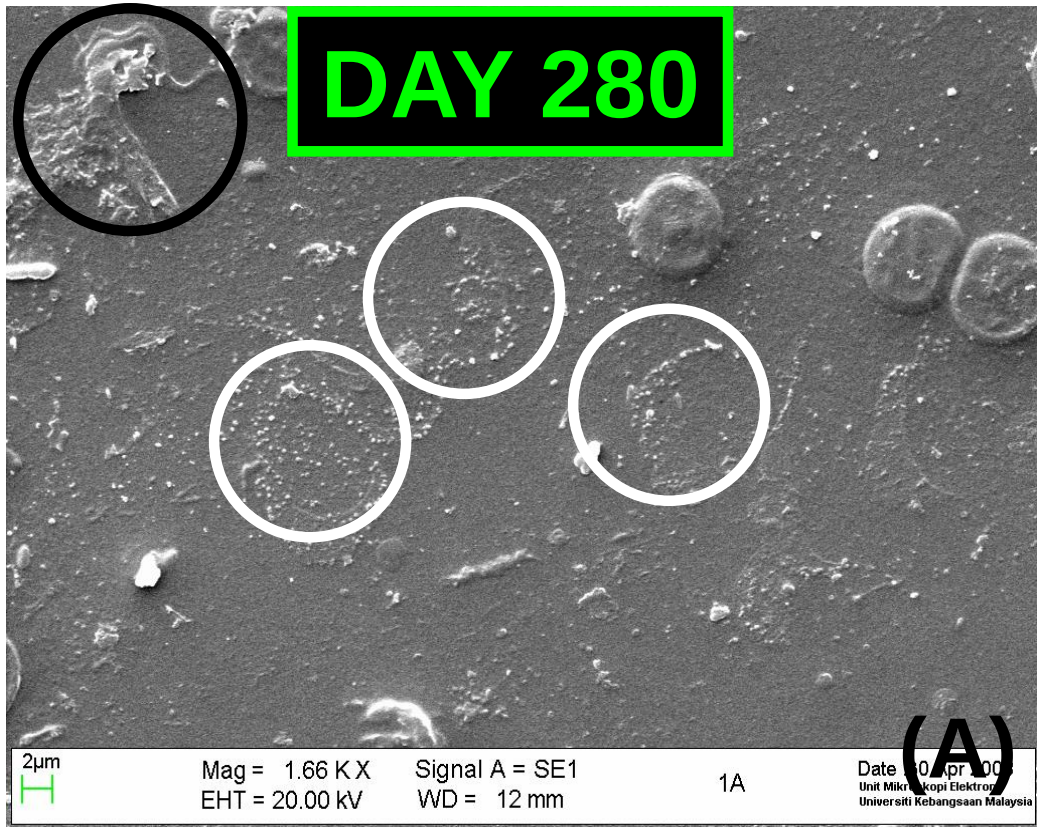
Parasite Growth & Survival in PRE14 Mice : Day 280 & 281

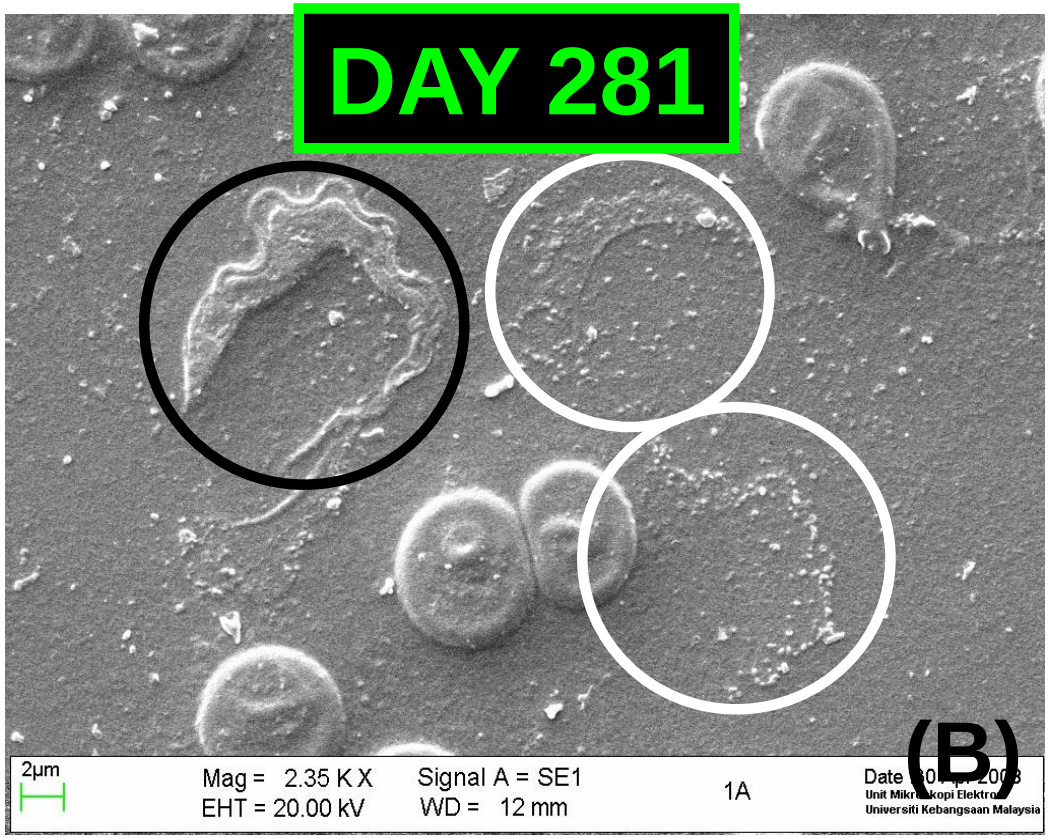


Reemerged of *T. evansi* which survived in PRE14 mice group on day 280 (A) and day 281 (B) due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as observed under x100 magnification of light microscope.

RESULTS & DISCUSSION

Parasite Growth & Survival in PRE14 Mice : Day 280 & 281

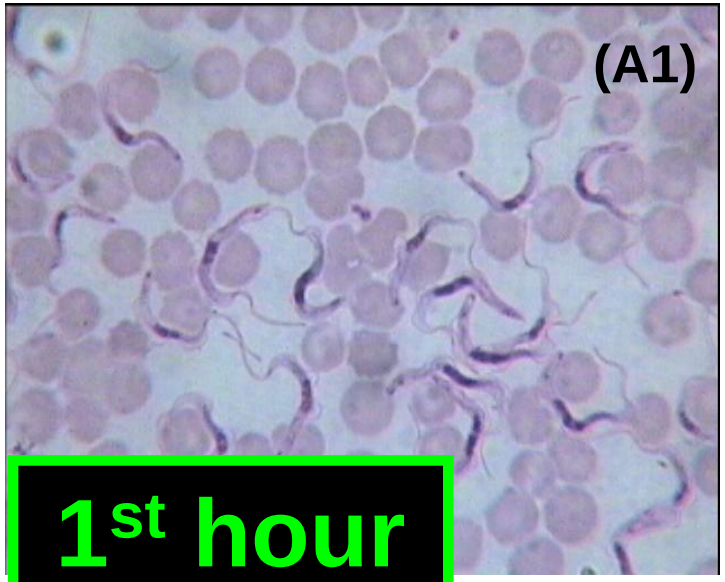




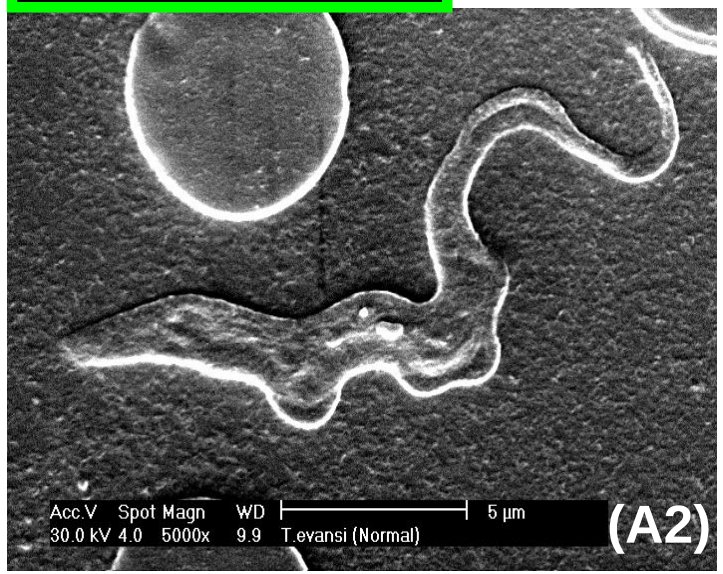
Reemerged of *T. evansi* which survived in PRE14 mice group on day 280 (A) and day 281 (B) due to the action of 'variable surface glycoprotein (VSA) stochastic genetic modification' as respectively observed under x1600 (A) and x2300 (B) magnification by SEM (Leo 1450VP, Japan).

RESULTS & DISCUSSION

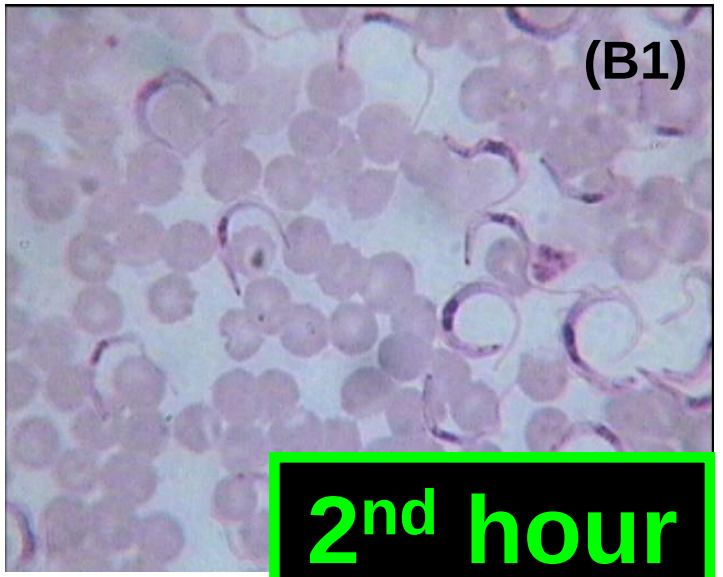
Parasite Growth & Survival in POS Mice : 1st & 2nd hour



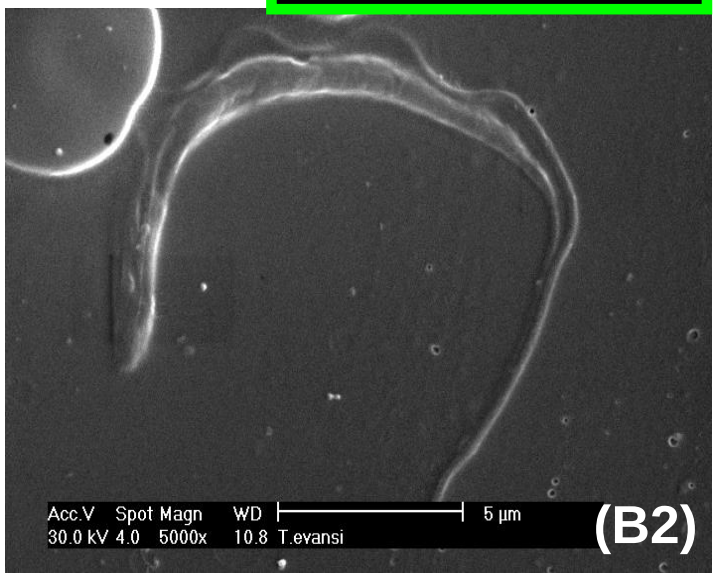
1st hour



The growth of *T. evansi* in POS mice group taken on 1 hour (left) and 2 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and x5000 magnification of SEM electron microscope (Phillips XL30, UK) (A2 & B2)

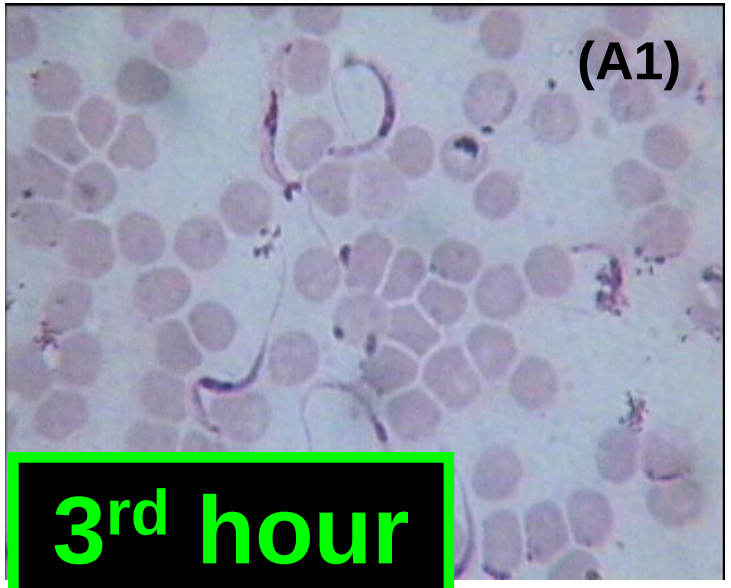


2nd hour

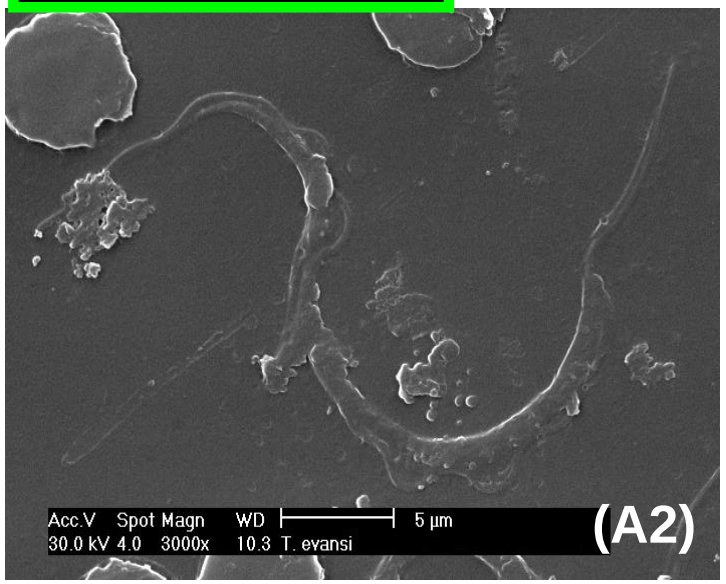


RESULTS & DISCUSSION

Parasite Growth & Survival in POS Mice : 3rd & 4th hour

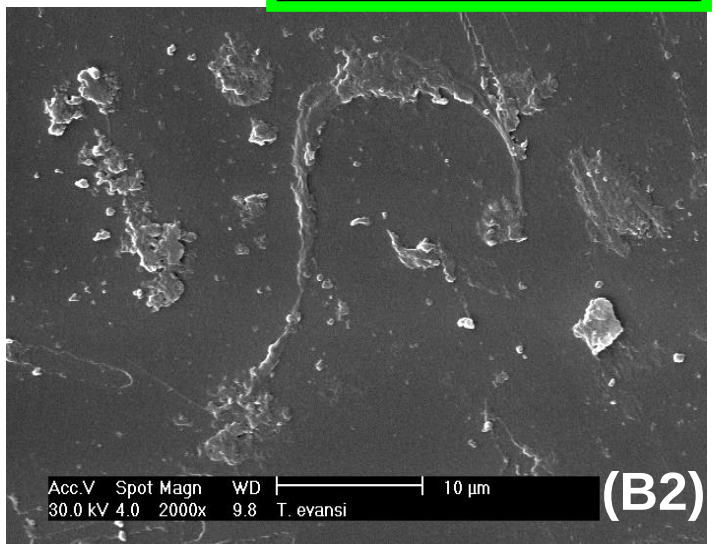


3rd hour



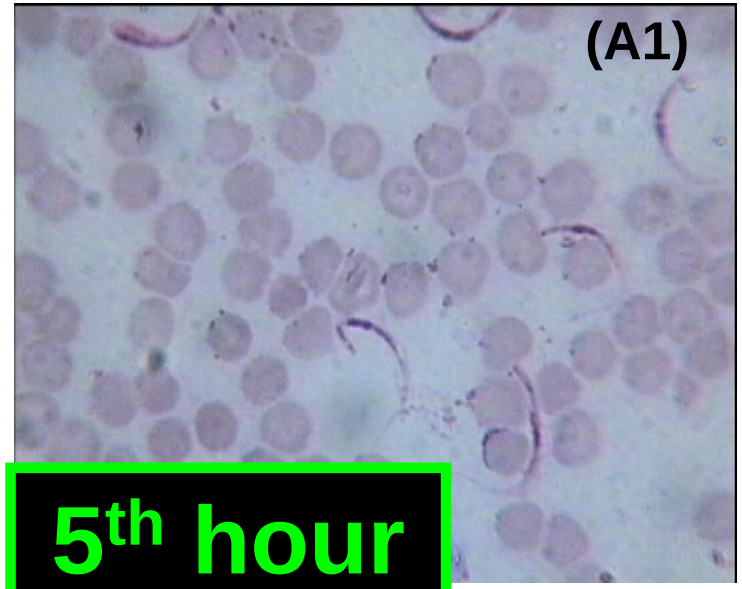
The growth of *T. evansi* in POS mice group taken on 3 hours (left) and 4 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and respectively under x3000 (A2) and x2000 (B2) magnification of SEM electron microscope (Phillips XL30, UK)

4th hour

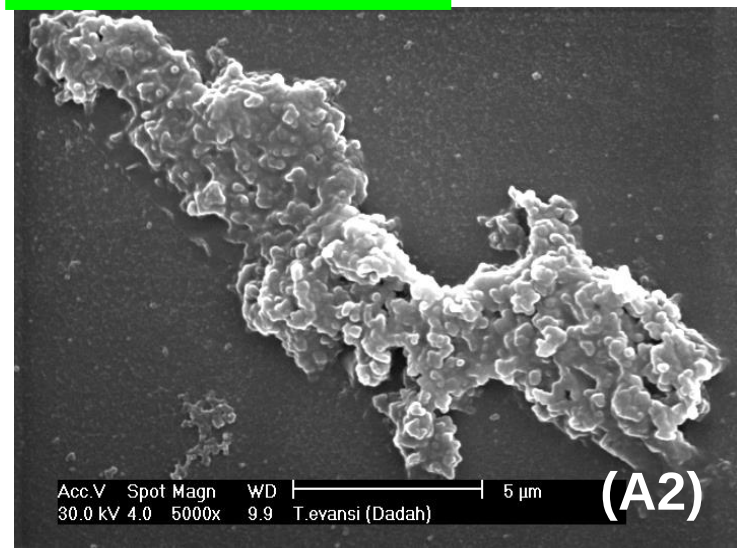


RESULTS & DISCUSSION

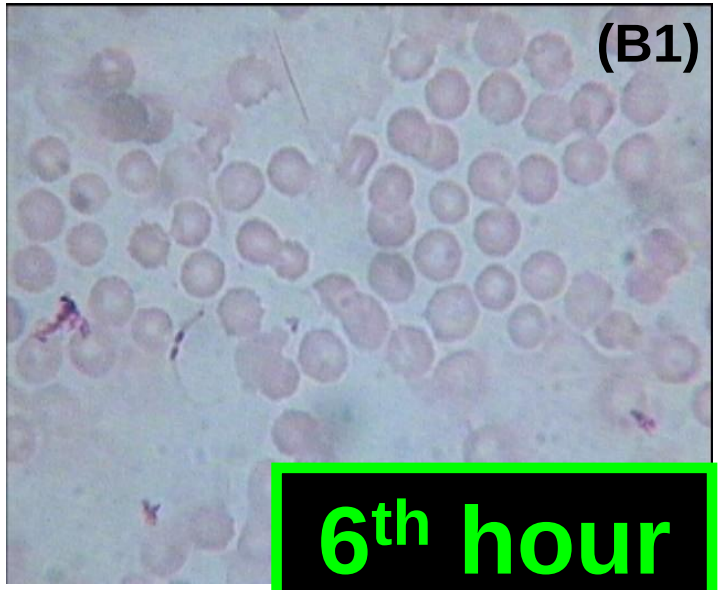
Parasite Growth & Survival in POS Mice : 5th & 6th hour



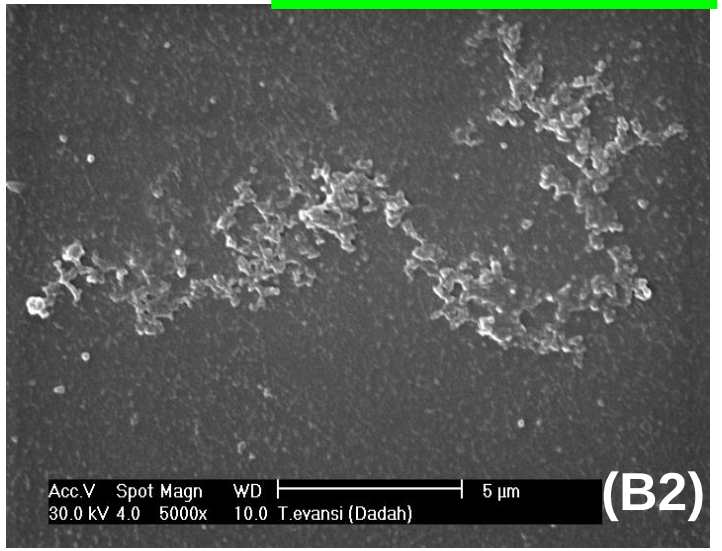
5th hour



The growth of *T. evansi* in POS mice group taken on 5 hours (left) and 6 hours (right) post-treatment (0.01 mL 3.5 mg/kg bw Berenil) as observed under x100 magnification of light microscope (AI & B1) and x5000 magnification of SEM electron microscope (Phillips XL30, UK) (A2 & B2)

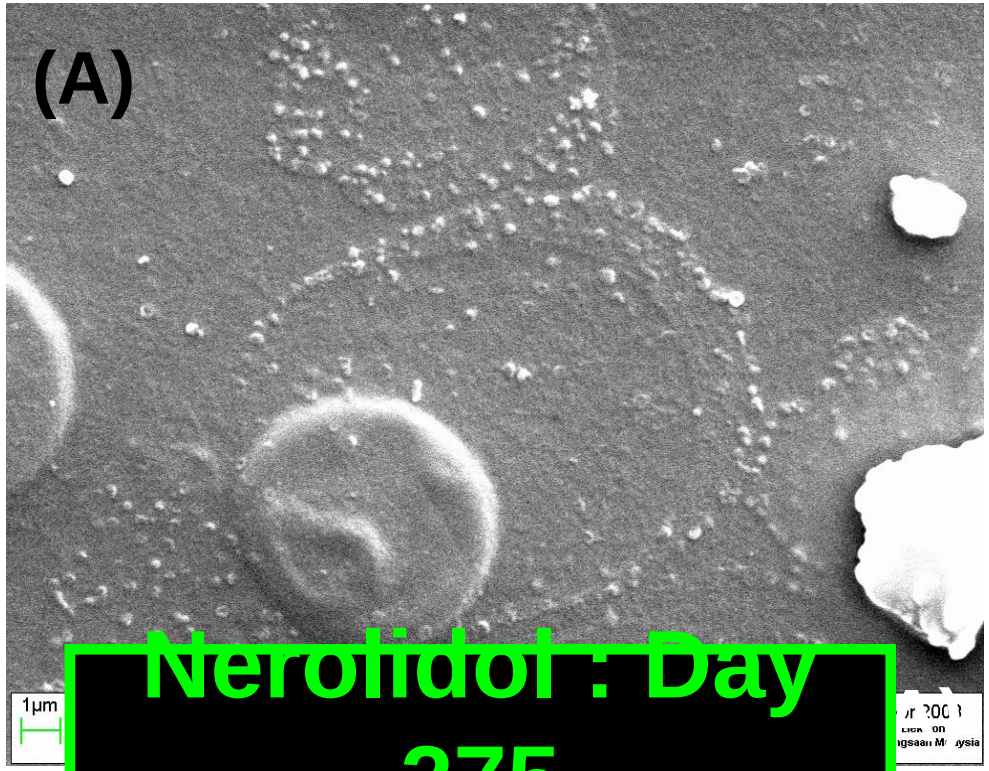


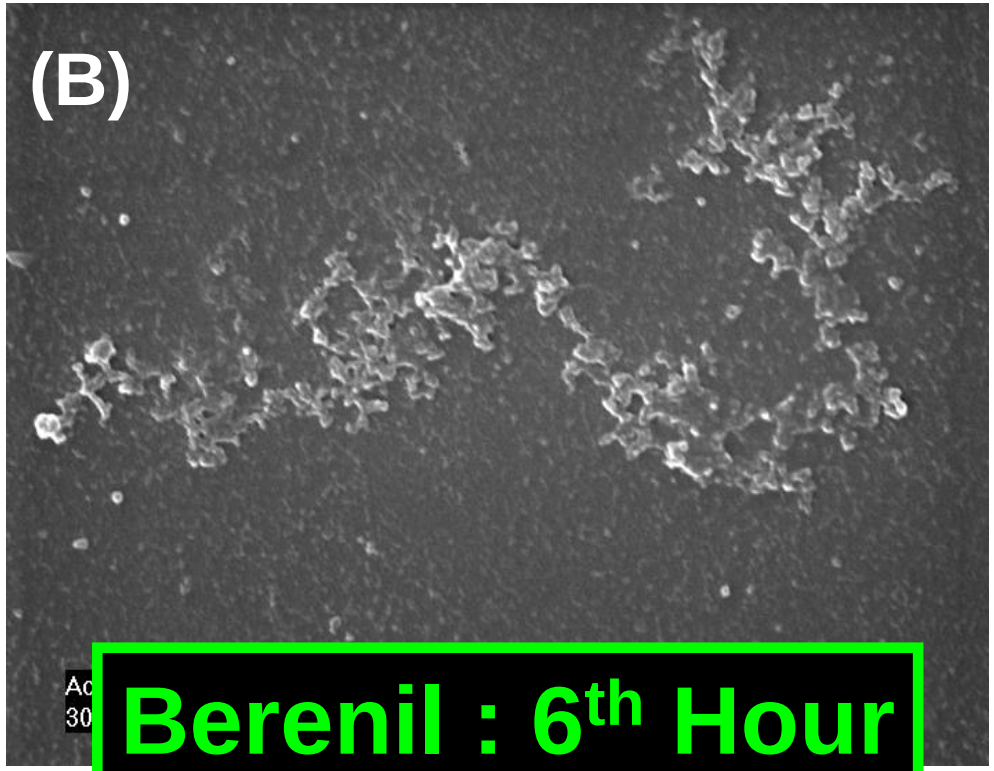
6th hour



RESULTS & DISCUSSION

Parasite Growth & Survival Against Nerolidol vs Berenil

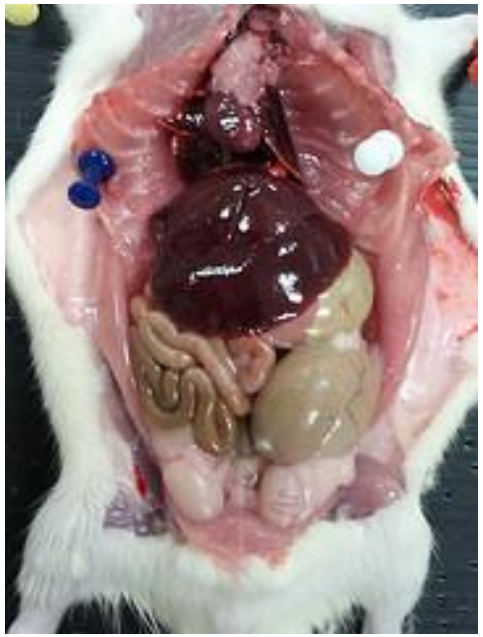




SEM micrograph showed the morphological changes of *T. evansi* in PRE14 mice on 275th day post infection (x5000, Leo 1450VP, Japan), 7 days just before the mice die (A) and in POS mice at 6th hours post infection (x5000, Phillips XL30, UK) (B)

RESULTS & DISCUSSION

Biochemical Tests For In-Vivo Toxicity Assessment



	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

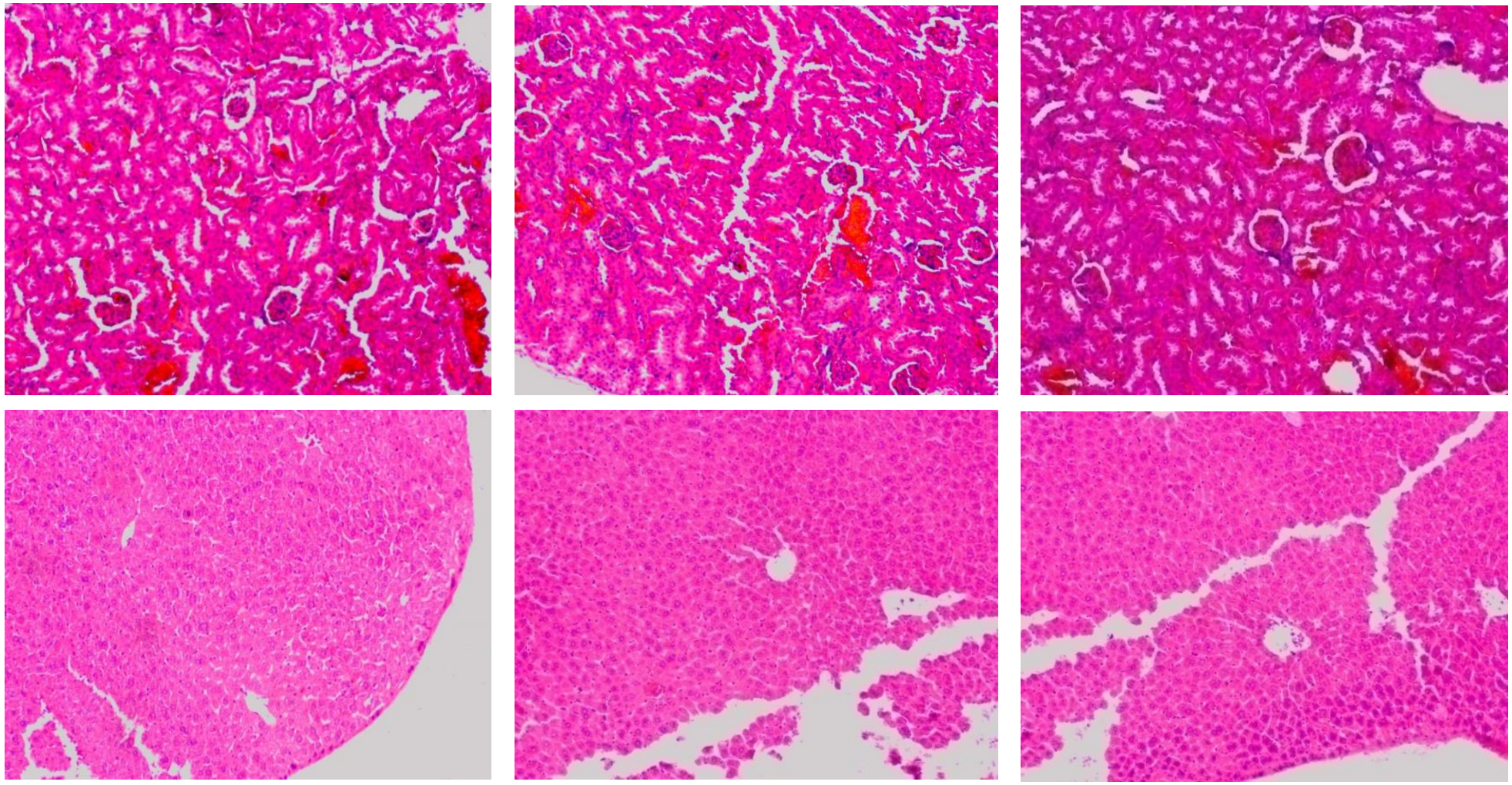
TA : Sub-acute regime – Daily treatment (28 days)
TB : Sub-acute regime – Daily treatment (28 days) 2 hours post-infection
TC : Sub-chronic regime – Daily treatment (90 days)
TD : Sub-chronic regime – Daily treatment (90 days) 2 hours post-infection
CN : Control regime – Normal mice without infection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

RESULTS & DISCUSSION

Organ Histology For Toxicity Assessment

KIDNEY

LIVER



Treatment (Acute)

Treatment (Sub-acute)

Control

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CONCLUSIONS

CONCLUSIONS

CONCLUSION

Hypothesis

- Stochastic genetic modification of VSG is still the best weapon for trypanosome survival (Nok et al. 1996).
- New wave of infection → mice is susceptible to infection (Ogunlana et al. 1944)
- Generally, nerolidol able to prolong the survival rate of the mice although the parasitemia density is quite high
- Significantly, curative regime are less effective than other treatment regimens
- Longer prophylactic duration → longer survival rate of the patient although the parasitemia has reached almost 50%.
- Alteration of dosage, concentration and period of prophylactic treatment → significant antiparasitic activity of nerolidol

CONCLUSION

Suggestions & Future Directions

1. Mechanisms of actions & molecular approaches
2. Extract of *E. cardamomum* daily doable for consumer
3. Play around with different concentration & dosage
4. To deal with stochastic genetic modification of VSG
5. Screening against *T. cruzi*, *T. brucei*, *Leishmania* spp. etc..
6. Holistic OH approach towards multidisciplinary collaboration
   → veterinary & medical doc, parasitologist, entomologist, botanist, zoologist, geologist, chemist, educationist, policy makers & politicians, local & global public, etc..

REFERENCES

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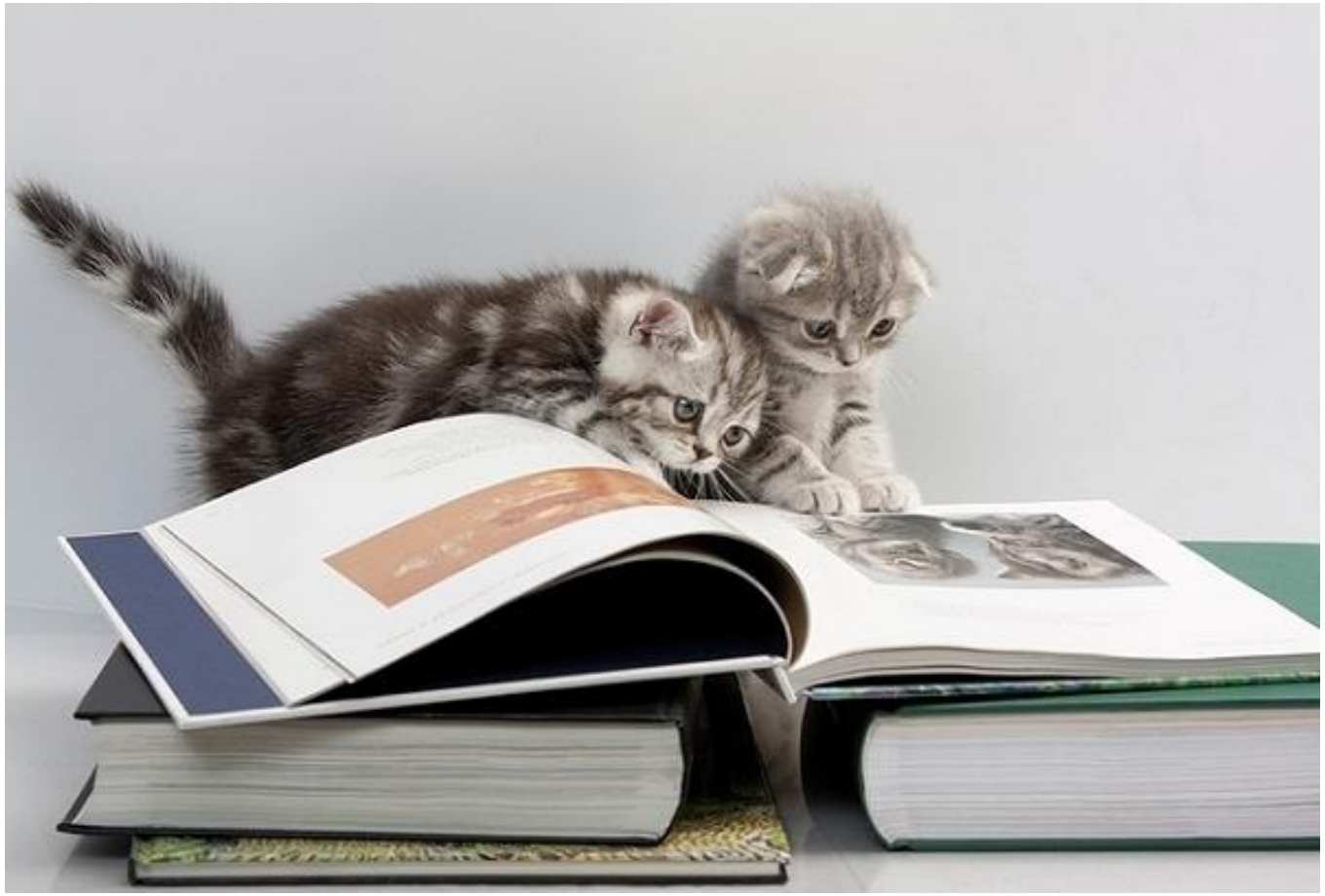

LES RÉFÉRENCES

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REMERCIE

‘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