



*Mahidol University: Wisdom of the Land*

# **Toxoplasmic Encephalitis: the Use of Stage-Specific Gene as a Diagnostic Tool**

**Yaowalark Sukthana**

**Department of Protozoology, Faculty of Tropical Medicine  
Mahidol University, Bangkok, THAILAND**



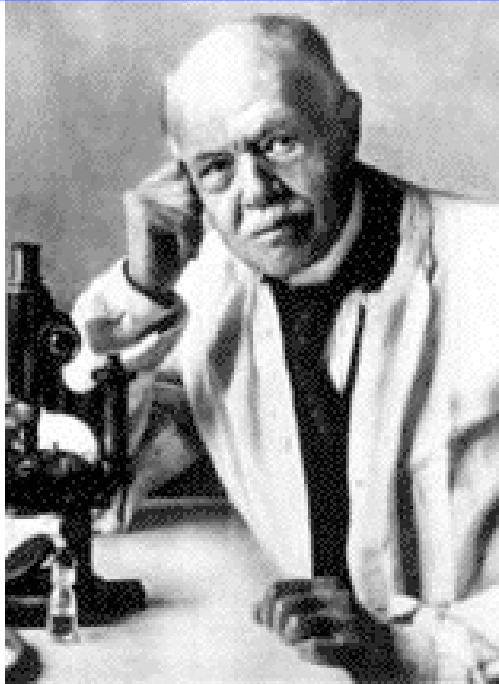
# Contents

- A Centennial *Toxoplasma gondii*
- Toxoplasmic encephalitis
- Diagnostic Review
- The way ahead





# A Centennial *T. gondii* (1908-2008)



**Charles J.H. Nicolle**  
(1866-1936)

**Louis Hubert Manceaux**  
(1865-1943)



*Ctenodactylus gundi*



**Alfonso Splendore**  
(1871 – 1953)



# A last century of *T. gondii*

- 1908: Nicholle & Mancaux; Splendore
- 1923: Janku reported chorioretinitis
- 1940: Pinkerton & Weinman reported disseminated toxoplasmosis
- 1948: Sabin & Feldman
- 1969: Dubey & Frenkel-Life cycle
- 1970s: Congenital Toxoplasmosis
- 1980s: Toxoplasmosis in HIV/AIDS
- 2000s: Environmental Issue

# Sero-prevalence in humans



Mahidol University:  
Wisdom of the Land

**USA 22.5%**  
(Jones *et al.*, 2001)

**France up to 75%**  
(Tenter *et al.*, 2000)

**Korea 6.9%**  
(Lee *et al.*, 2000)

**Taiwan 23%**  
(Fan *et al.*, 2002)

**Vietnam 20%**  
(Sery *et al.*, 1988)

**Thailand 12.4%**  
(Sukthana *et al.*, 2000)

**Indonesia 70%**  
(Terazawa *et al.*, 2003)

**Brazil 73.3%**  
(Cavalcante *et al.*, 2006)

**Malaysia 20-30%**  
(Nissapatorn *et al.*, 2004)

**Africa 71.43%**  
(Lopez *et al.*, 1992)

**Australia 30-40%**  
(Johnson *et al.*, 1980)



# A last century of *T. gondii*



**Martina Navratilova**

developed Acute  
Toxoplasmosis in 1982



**Arthur Ashe (1943-1993)**

Career prize US\$ 2,584,909  
Developed TE in 1988



# Transmission

**Definite Host**

**External Environment**

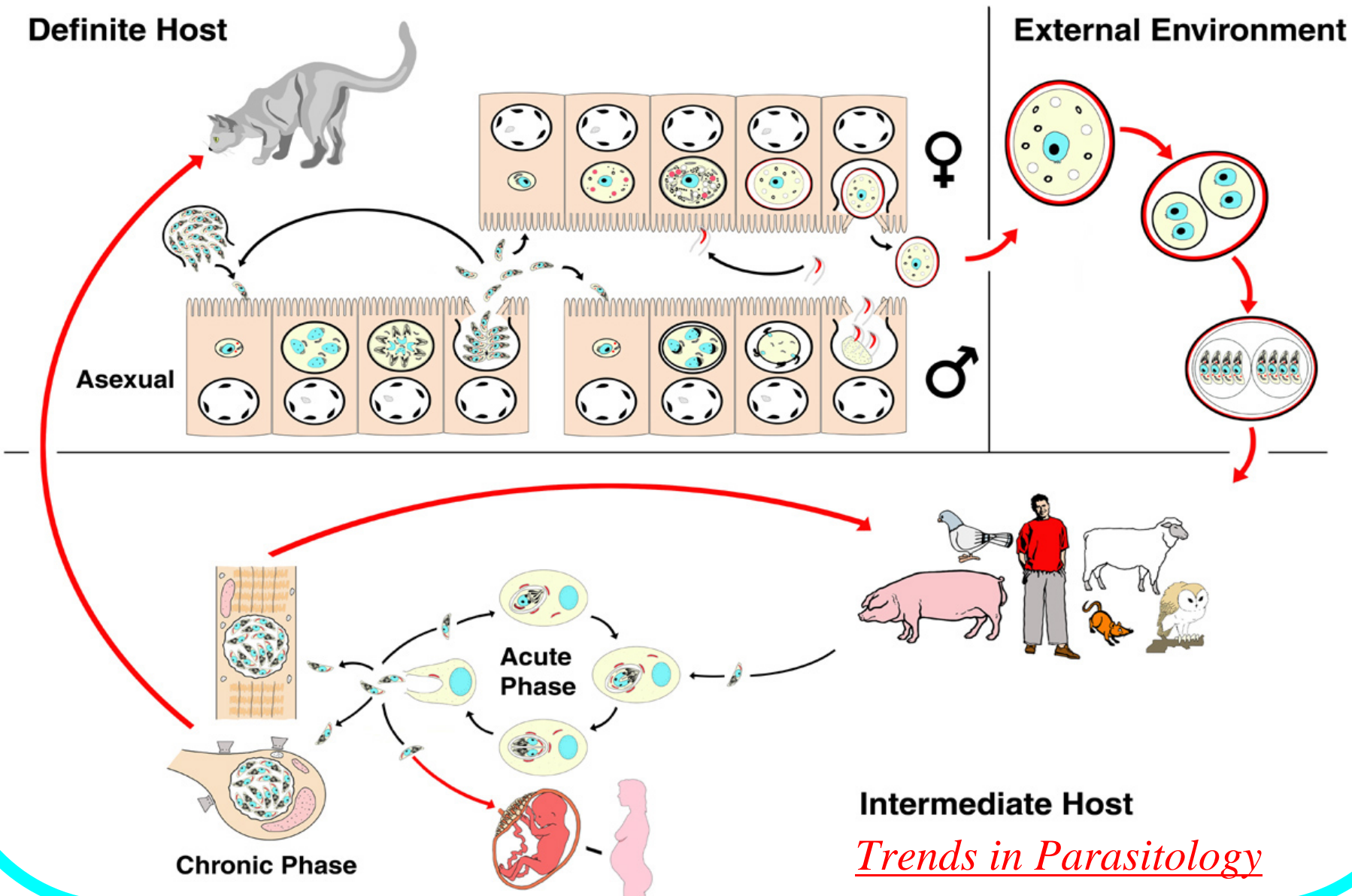
**Asexual**

**Acute Phase**

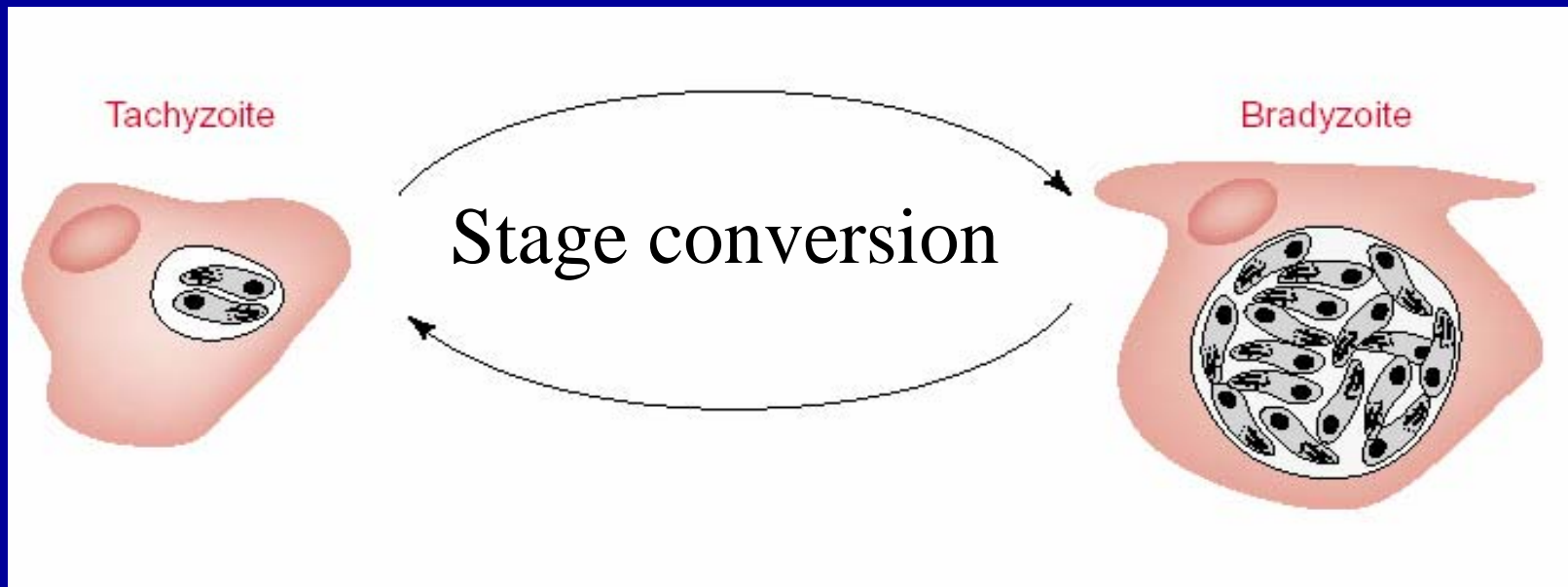
**Chronic Phase**

**Intermediate Host**

*Trends in Parasitology*







- **Acute phase**
- **Proliferate in many cells including macrophages**
- **Destroyed by immune response**
- **Sensitive to drug treatment**
- **Chronic phase**
- **Cysts in brain and muscles**
- **Viable for life of host**
- **Resistant to drug treatment**
- **Source of infection in the immunocompromised**

(Lyons *et al.*, 2002 from *Trends in Parasitology* )





# Toxoplasmic Encephalitis: TE

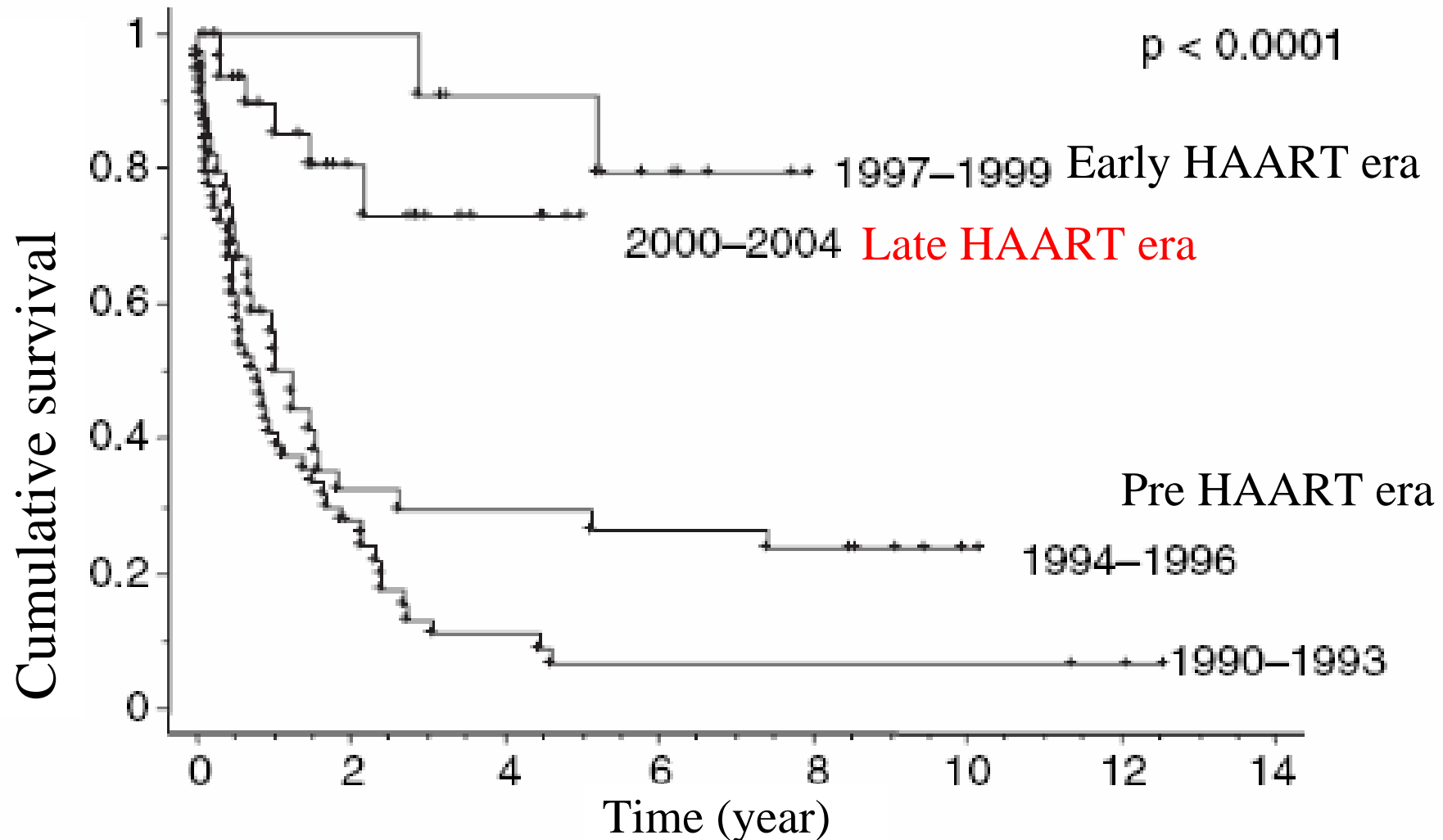
- In the early 1990s:  
TE annual incidence ~ 0.4-0.7 events per 100 person-year
- In 1992-1996: prophylactic regimens
- Since 1997: HAART era

---

(Bacellr et al, 1994, Ammassari et al, 1998; Belanger et al, 1999; Scaktor et al, 2001, Antinori et al, 2004)



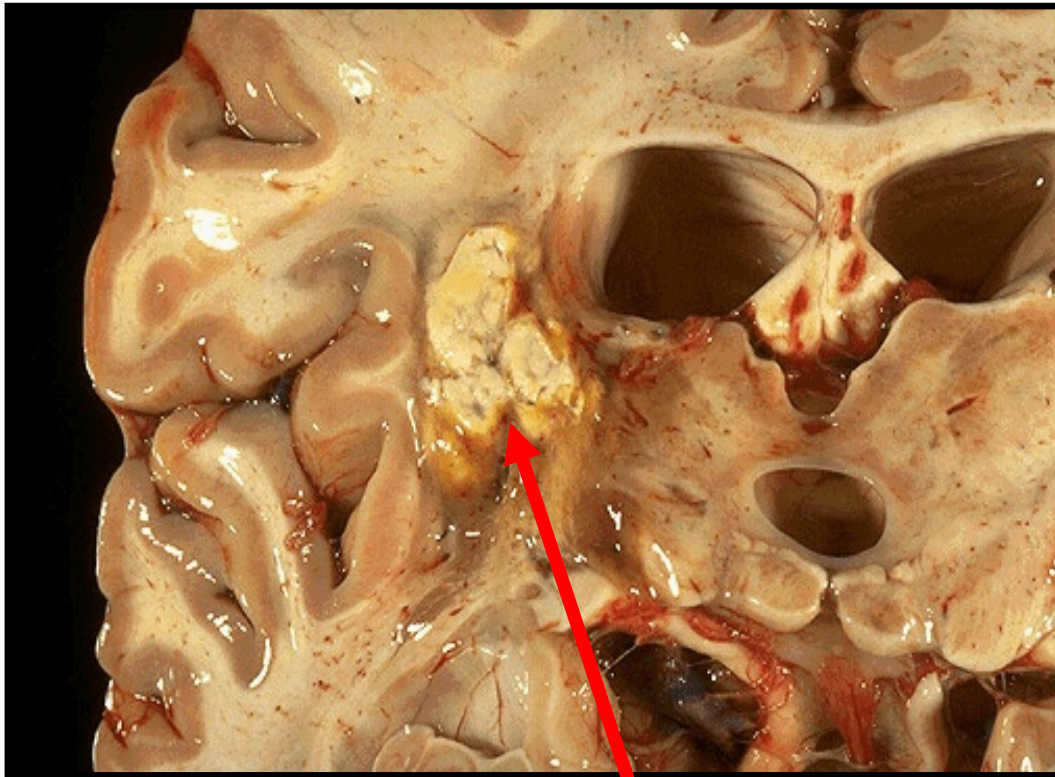
# Toxoplasma Encephalitis: TE



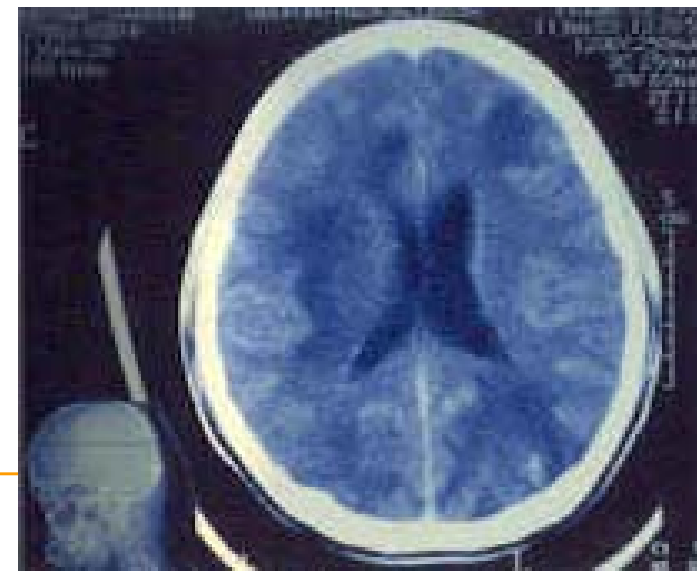
(Hoffmann et al, 2007)



# Toxoplasma Encephalitis: TE



**Toxoplasma abscess in  
the Brain**



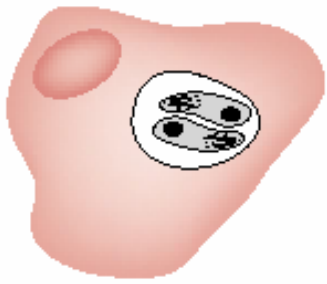
Sukthana, 2006; Mortier, 1996; CDC 1993





# Stage specific antigens

Tachyzoite



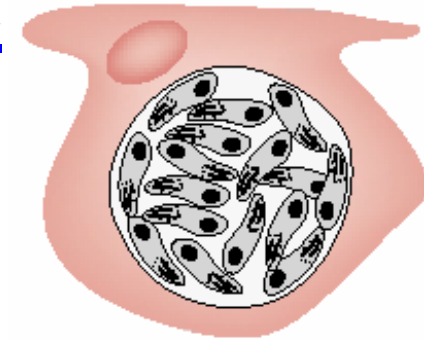
## Tachyzoite

- SAG1
- SAG2A
- SAG2B
- LDH1
- ENO2

## Bradyzoite

- BAG1
- MAG1
- SAG2C
- SAG2D
- BSR4
- LDH2
- ENO1

Bradyzoite



(Ferguson, 2004)



# *SAG1* and *BAG1* Genes

- **RT-PCR** (reverse transcription-PCR) was developed to detect tachyzoite/ bradyzoite stage conversion expressions during immunosuppressed periods in mice
- Then apply the duplex RT-PCR for detection of stage specific *T. gondii* in **clinical specimens** of HIV/AIDS persons



# Materials & Methods (1)

25 cysts of  
*T. gondii* O'Toole strain

Orally



25 ICR mice

6 weeks

Control group

Treated group  
(Kang *et al*, 2006)

Sacrificed on the last  
day of experiment

Sacrificed at  
1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> weeks

The brain, heart, liver were taken for

Duplex RT-PCR





# Duplex RT-PCR

- ✚ Total RNA extraction [TRIzol (Invitrogen)]
- ✚ Synthesized cDNA [RevertAid™ M-MuL V Reverse Transcriptase (Fermentas) and Oligo(dT)-primers]
- ✚ PCR reaction with stage specific primers:  
*SAG1* and *BAG1* genes: Rachlin *et al*, 2005)

# Primers



Mahidol University:  
Wisdom of the Land

*SAG1*(AY651825) *BAG1* (Z48750) genes

muPlex program (Rachlin *et al*, 2005),  
1<sup>st</sup> BASE INC. (Singapore)

*SAG1S* 5' GCTGTAACATTGAGCTCCTTGATTTCCTG 3',

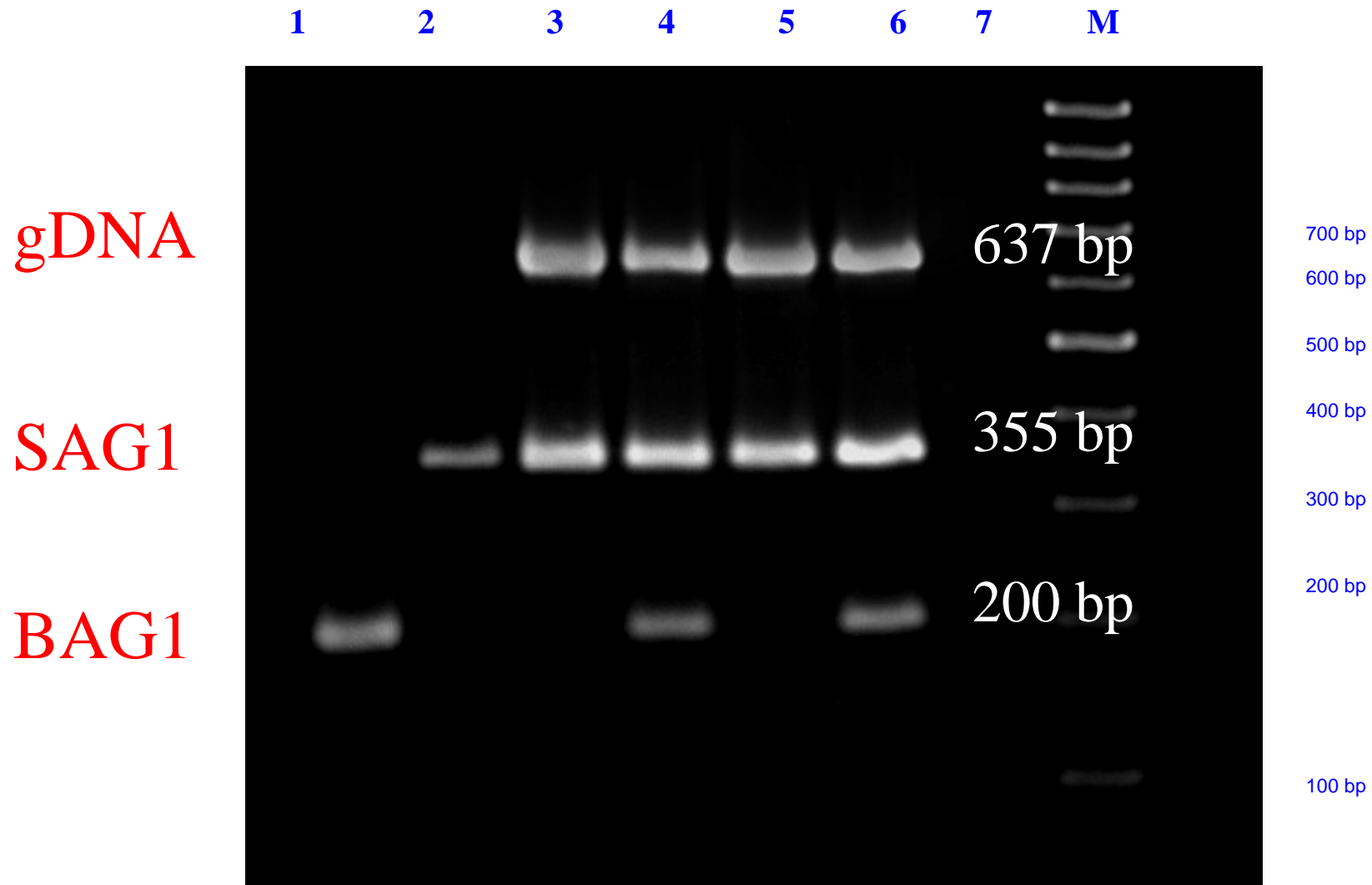
*SAG1AS* 5' CCGGAACAGTACTGATTGTTGTCTTGAG 3',

*BAG1S* 5' AGTCGACAACGGAGCCATCGTTATC 3', and

*BAG1AS* 5' ACCTTGATCGTGACACGTAGAACGC 3'



# Detection of amplified DNA







# Materials & Methods (2)

49 Clinical specimens from  
HIV/AIDS with CNS diseases

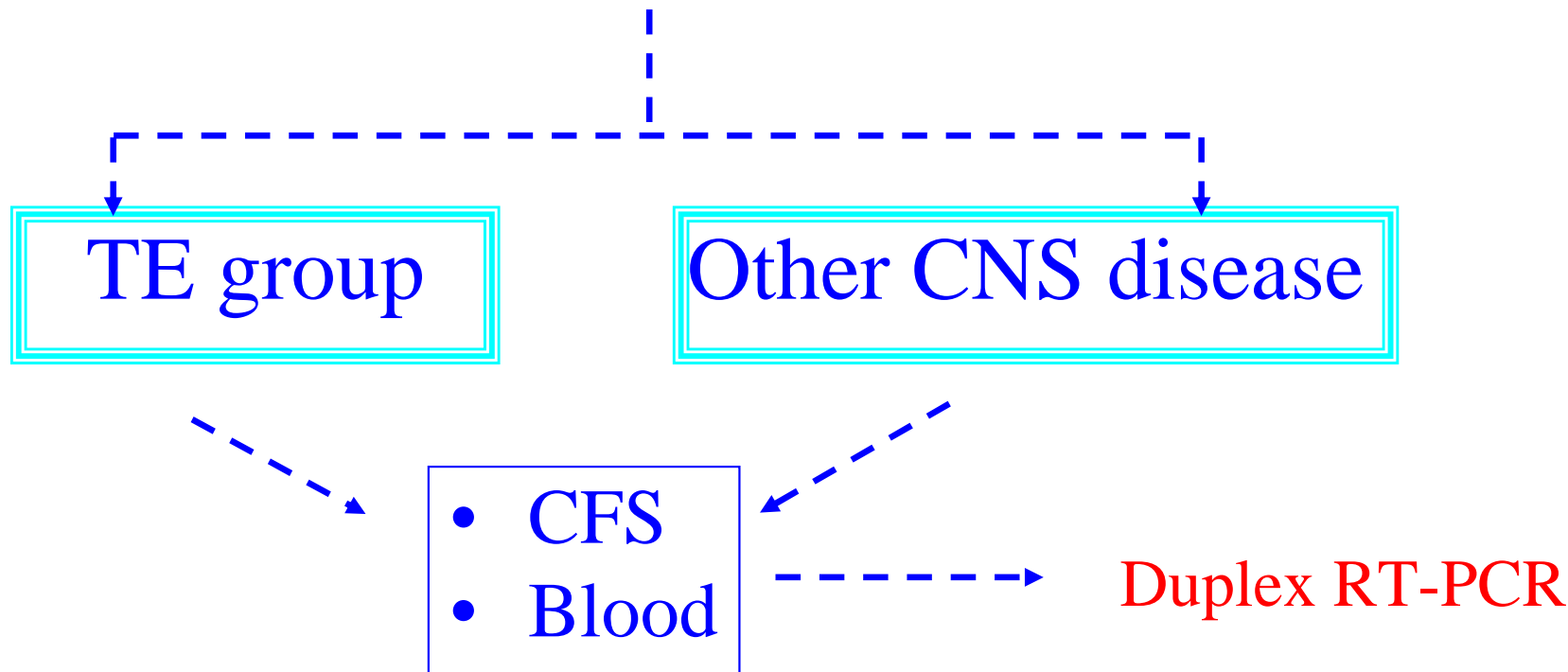
CDC criteria

TE group

Other CNS disease

- CFS
- Blood

Duplex RT-PCR





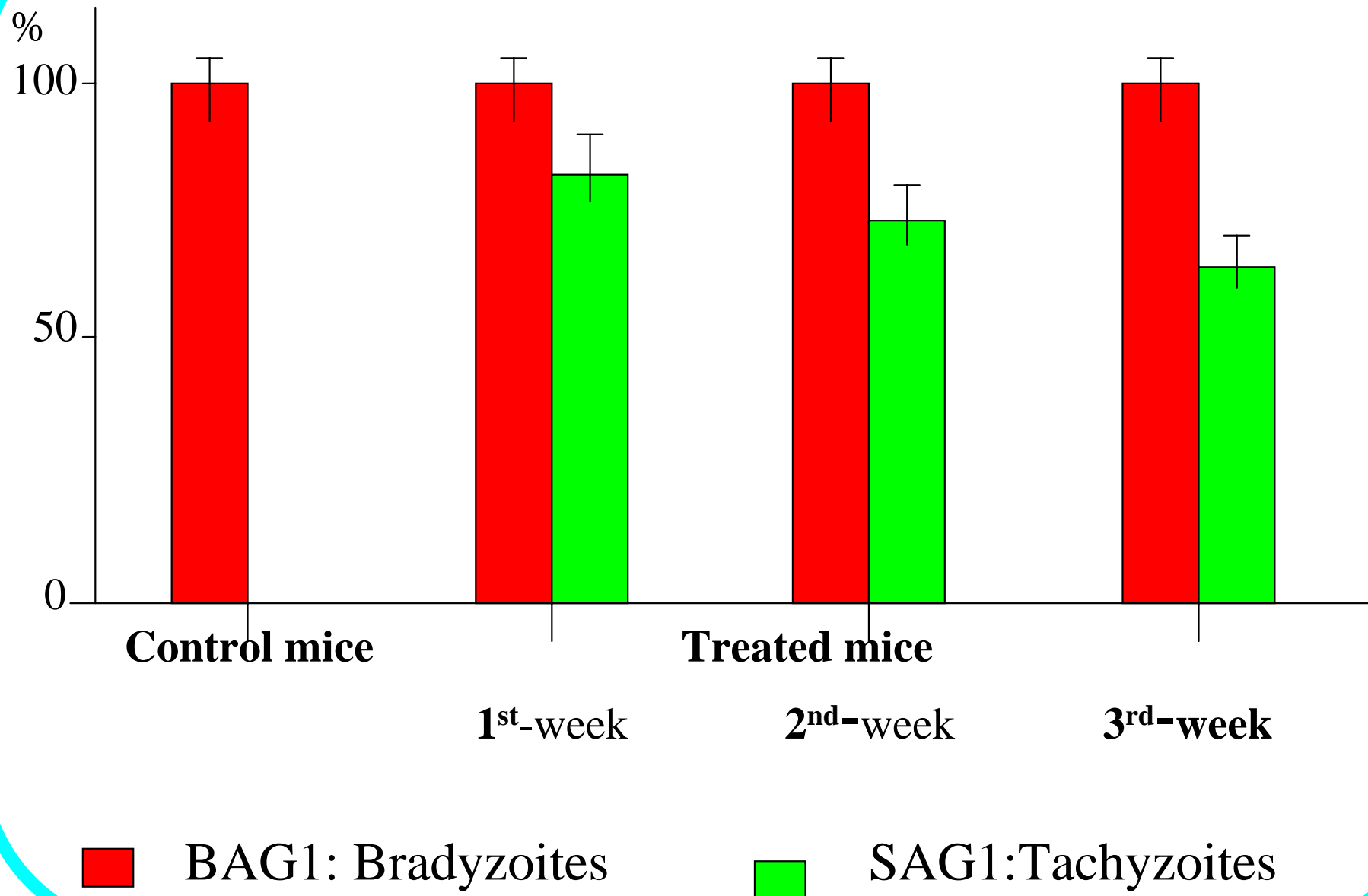
# Results (1)

- Experimental mice developed **illnesses**
- Some animals showed **neurological signs** as soon as 7 days after starting the suppression.
- In contrast, all control mice survived until the last day of the experiment without any clinical sign.

# RT-PCR results-Brain

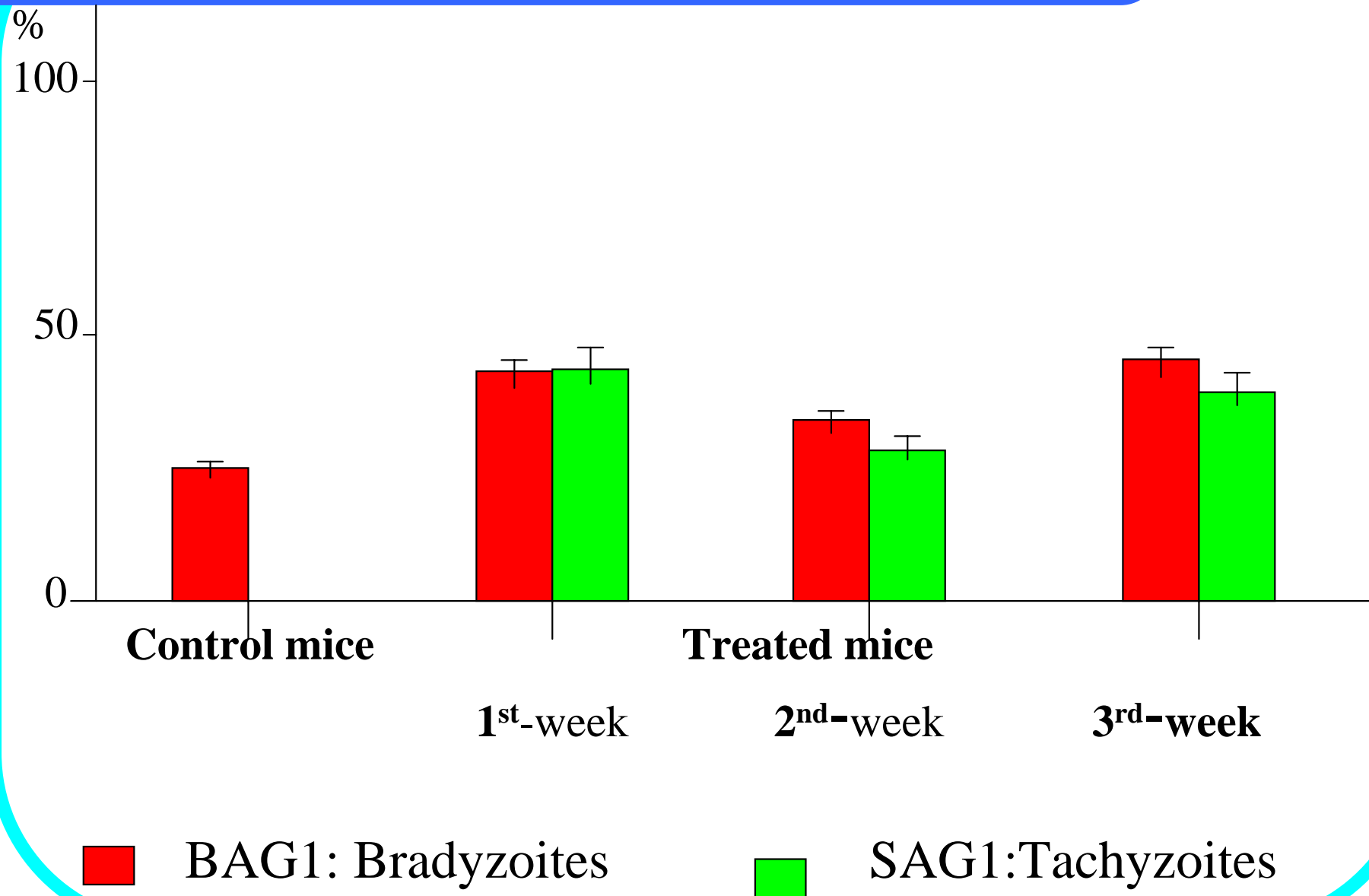


Mahidol University:  
Wisdom of the Land



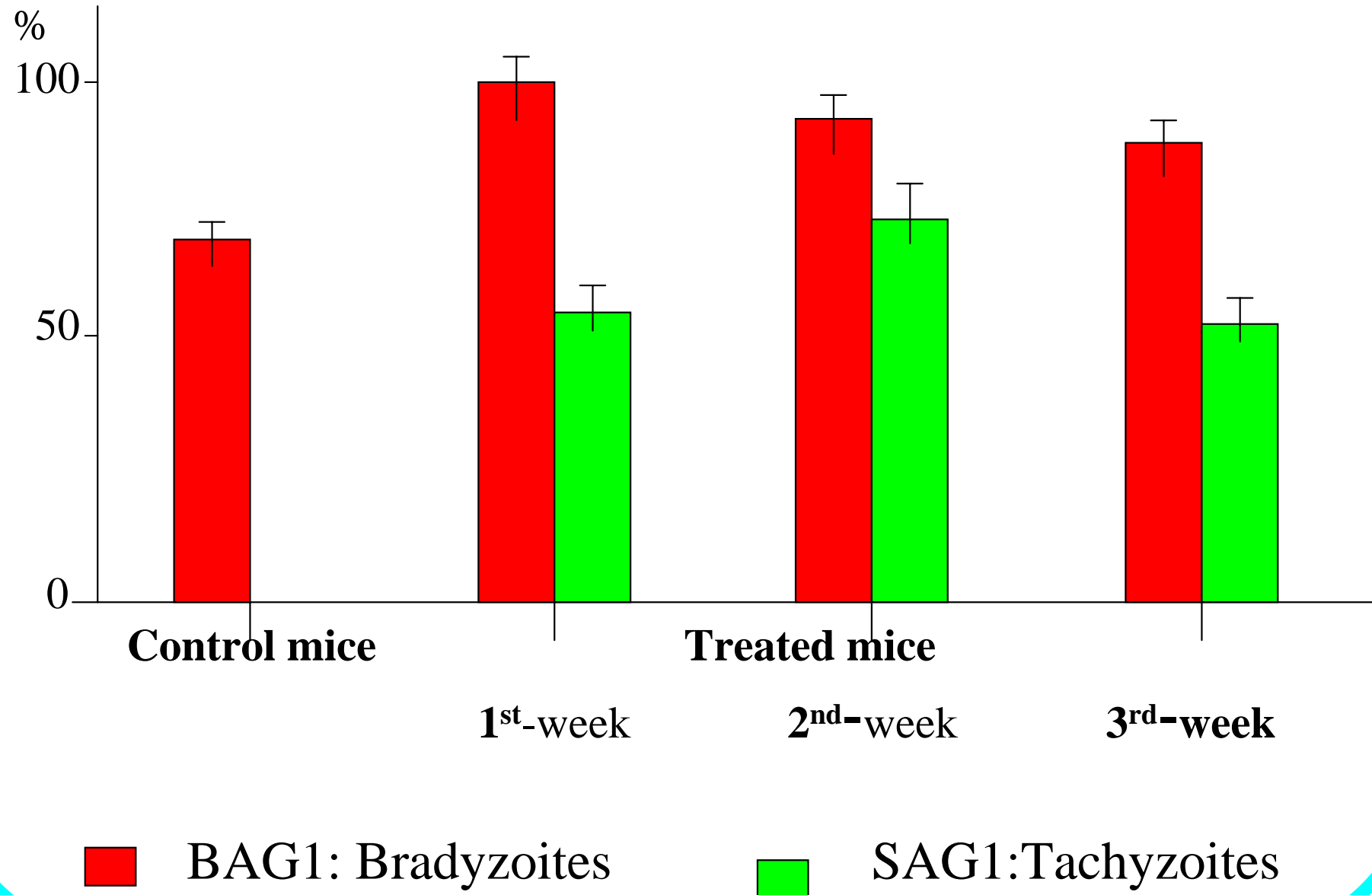


# Liver RT-PCR results





# Heart RT-PCR results



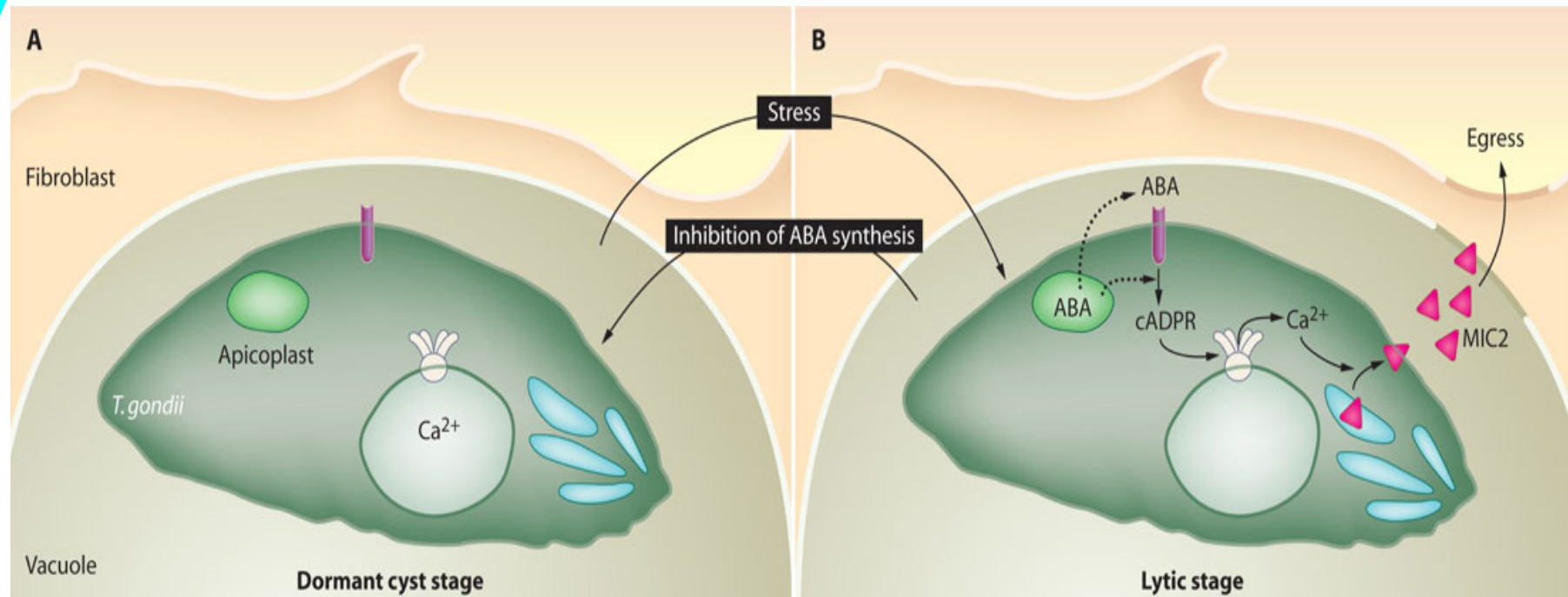


# Discussion



Mahidol University:  
Wisdom of the Land

## Regulation of *T. gondii* stage conversion



ABA= Abscisic acid, cADPR = cyclic adenosine diphosphoribose

Sibley and colleagues, 2008  
Reviewed by Guse, AH, 2008

Science Signaling

AAAS



# Discussion

- Bradyzoites egress from the cysts are not only reinvade neighboring cells as tachyzoites but also driven into new bradyzoites too.
- Moreover, since we found both stages co-existing in many organs, the reactivation is not a synchronous event.

(Gazzinelli *et al.*,1992 and Odeart *et al.*,1996)



# Results (2)

**9 out 49 positive**

**CDC**

**RT-PCR**

1	Case 15	✓	✓ B
2	Case 19	X	✓ T
3	Case 20	X	✓ B
4	Case 32	✓	✓ B
5	Case 33	✓	X B
6	Case 37	✓	✓ B
7	Case 38	✓	✓ B
8	Case 39	✓	✓ T
9	Case 41	✓	✓ B

Patients with TE  
(according to CDC criteria)

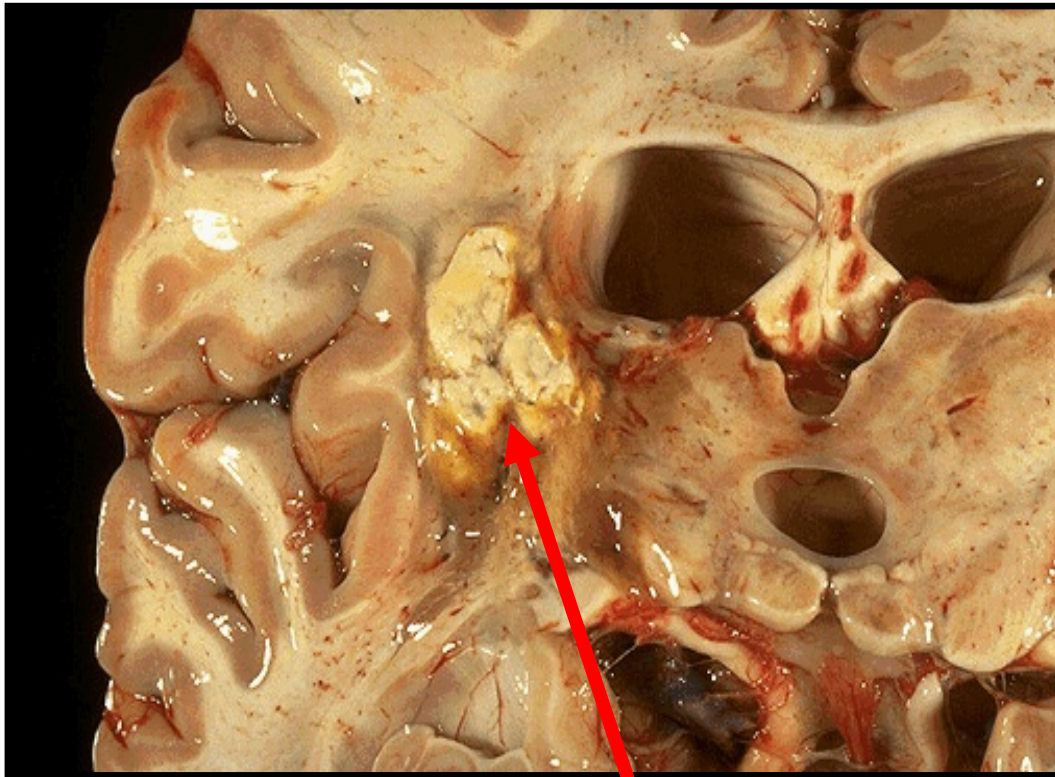
		Yes	No	Total
Duplex RT- PCR test	Positive	6	2	8
	Negative	1	40	41
	Total	7	42	49

Sensitivity = 86% : Specificity = 95%

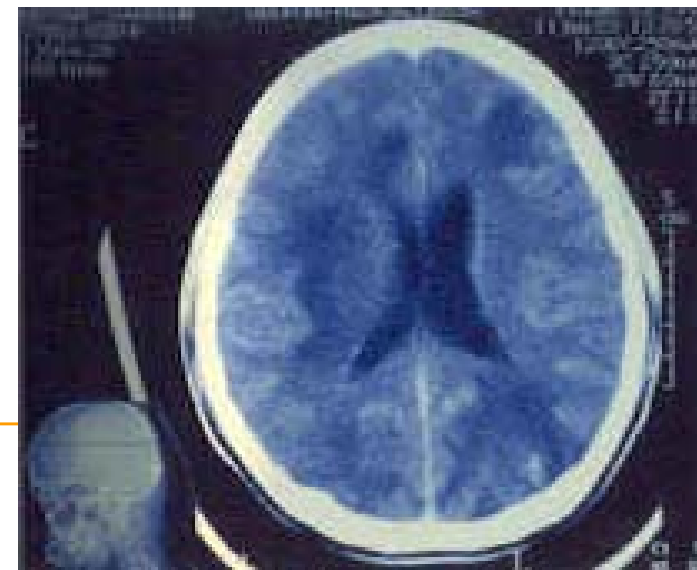
Positive Predictive Value = 75%

Negative Predictive Value = 98%

# Discussion



**Toxoplasmic encephalitis**



**Sukthana, 1999; 2006**





# Discussion

- RT-PCR with *SAG1* and *BAG1* genes provide information on the stages present which helpful in the prognosis and early diagnosis of TE.
- The better and appropriate prophylaxis or treatment could be administered.



# The Way Ahead

- A minimum of handling, or at best no handling, of the products is highly preferable.
- Real-time RT-PCR may circumvent this obstacle.



