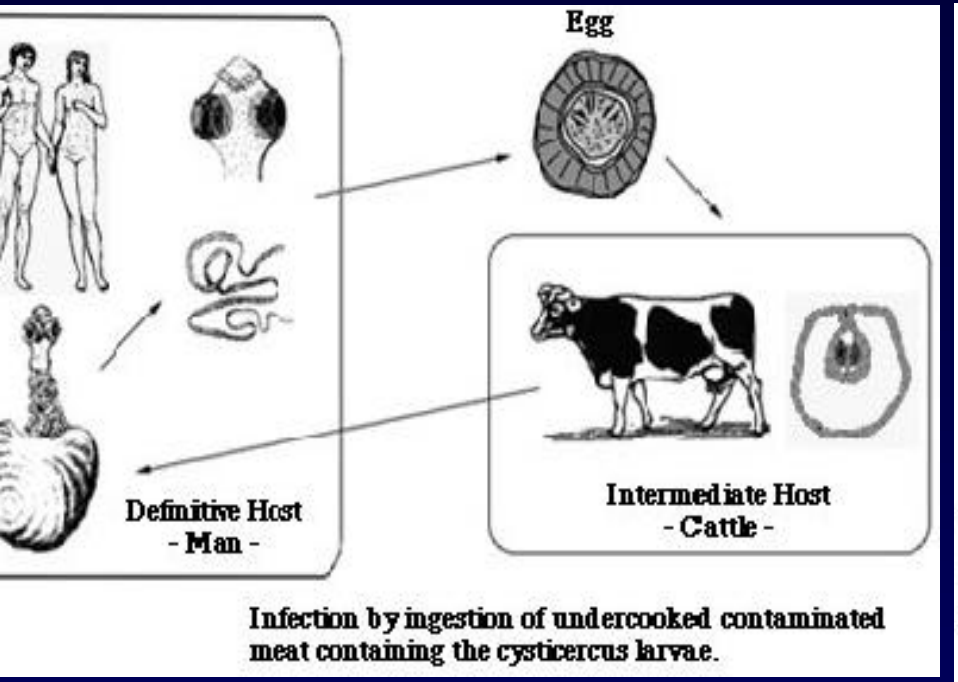
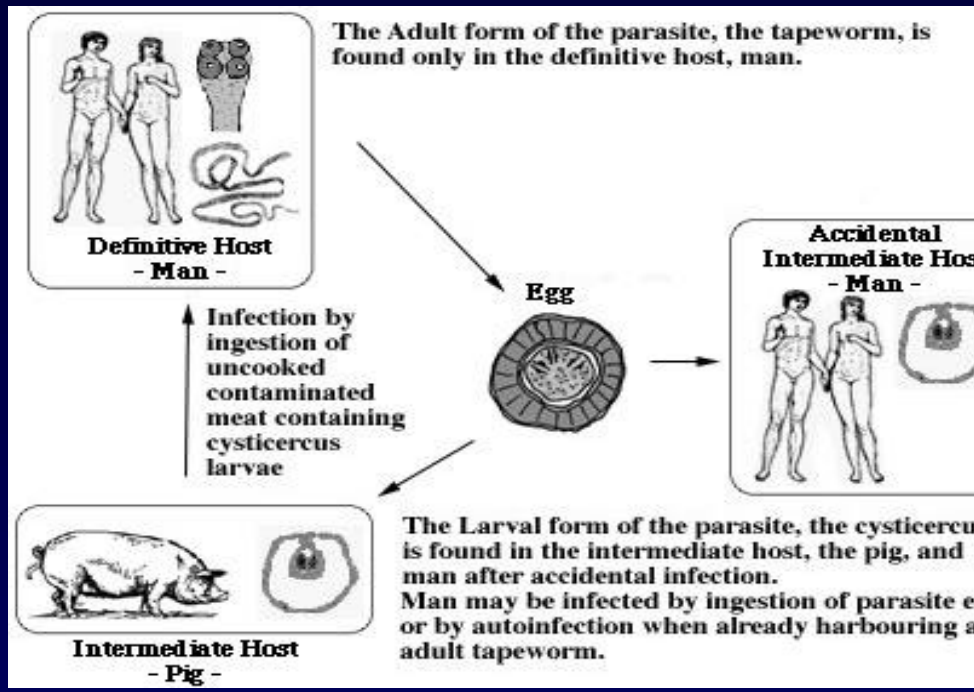




T. saginata

Beef tapeworm

T. solium

Pork tapeworm

T. solium is a homolactate fermentor, glycolysis stops at lactate

Glycolysis

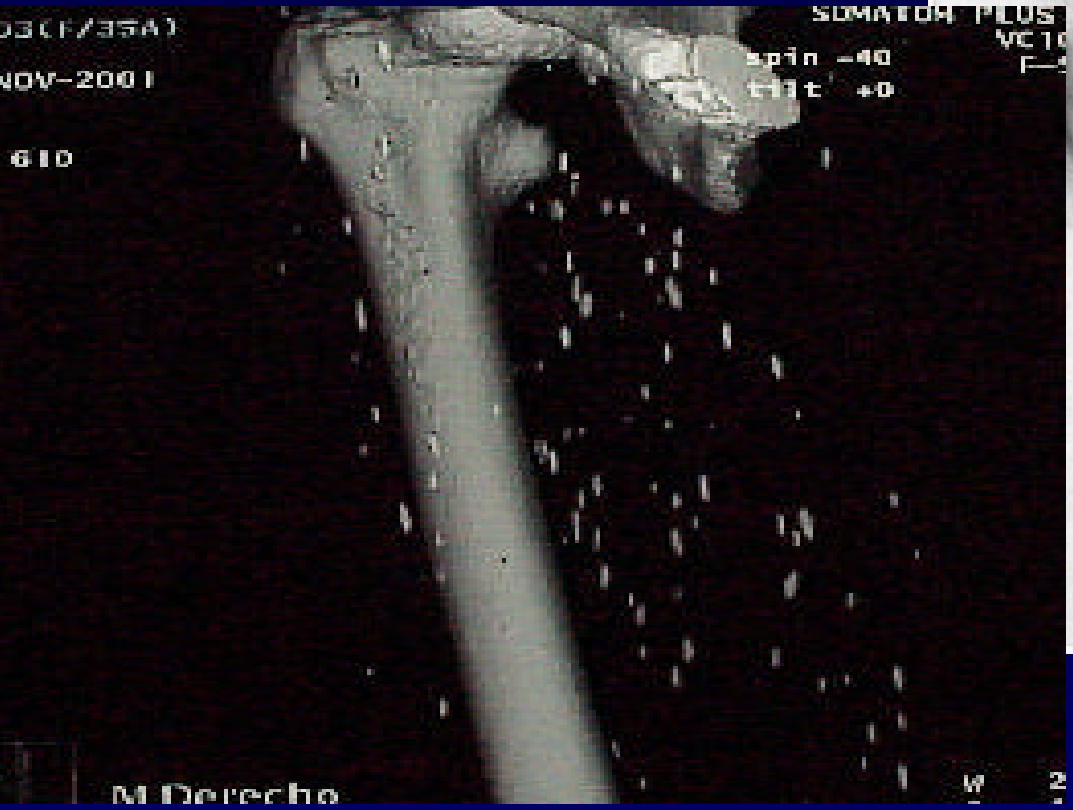
2 ATP + 2 NADH

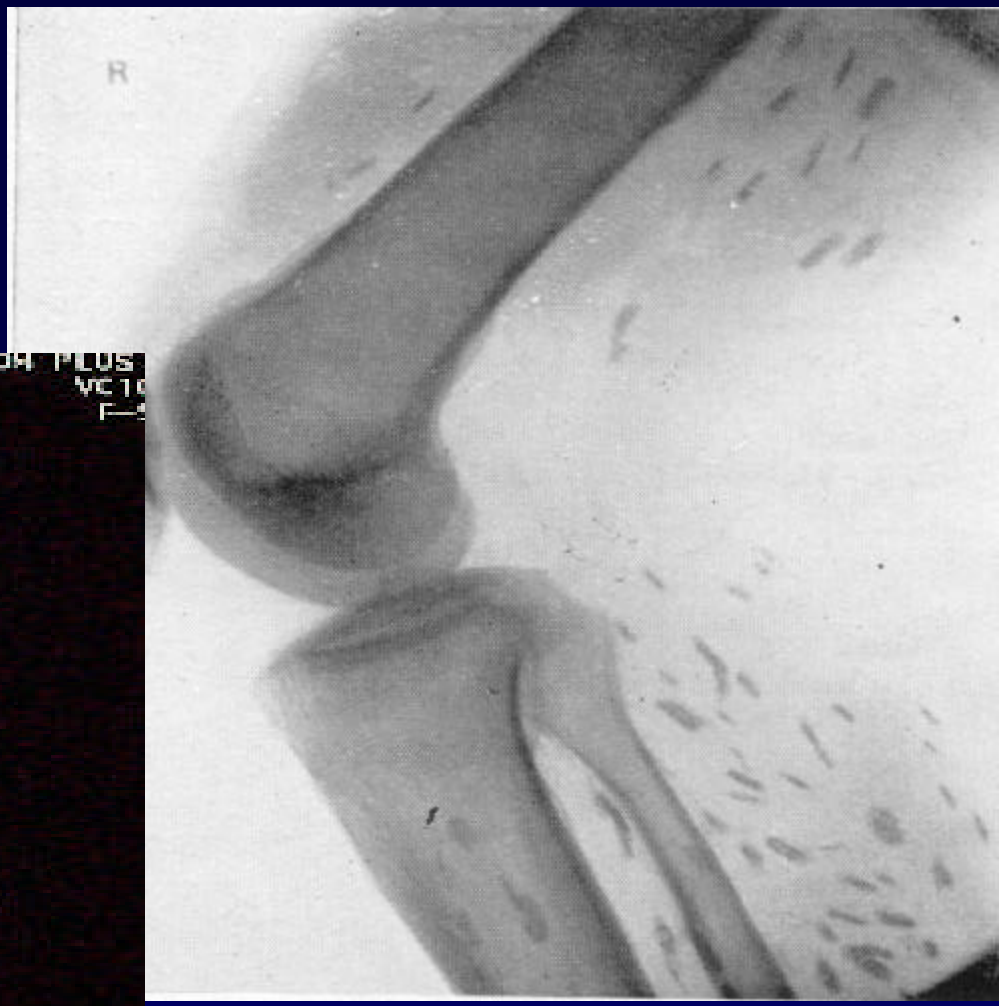
==========the bum stops here=(lactate)==========

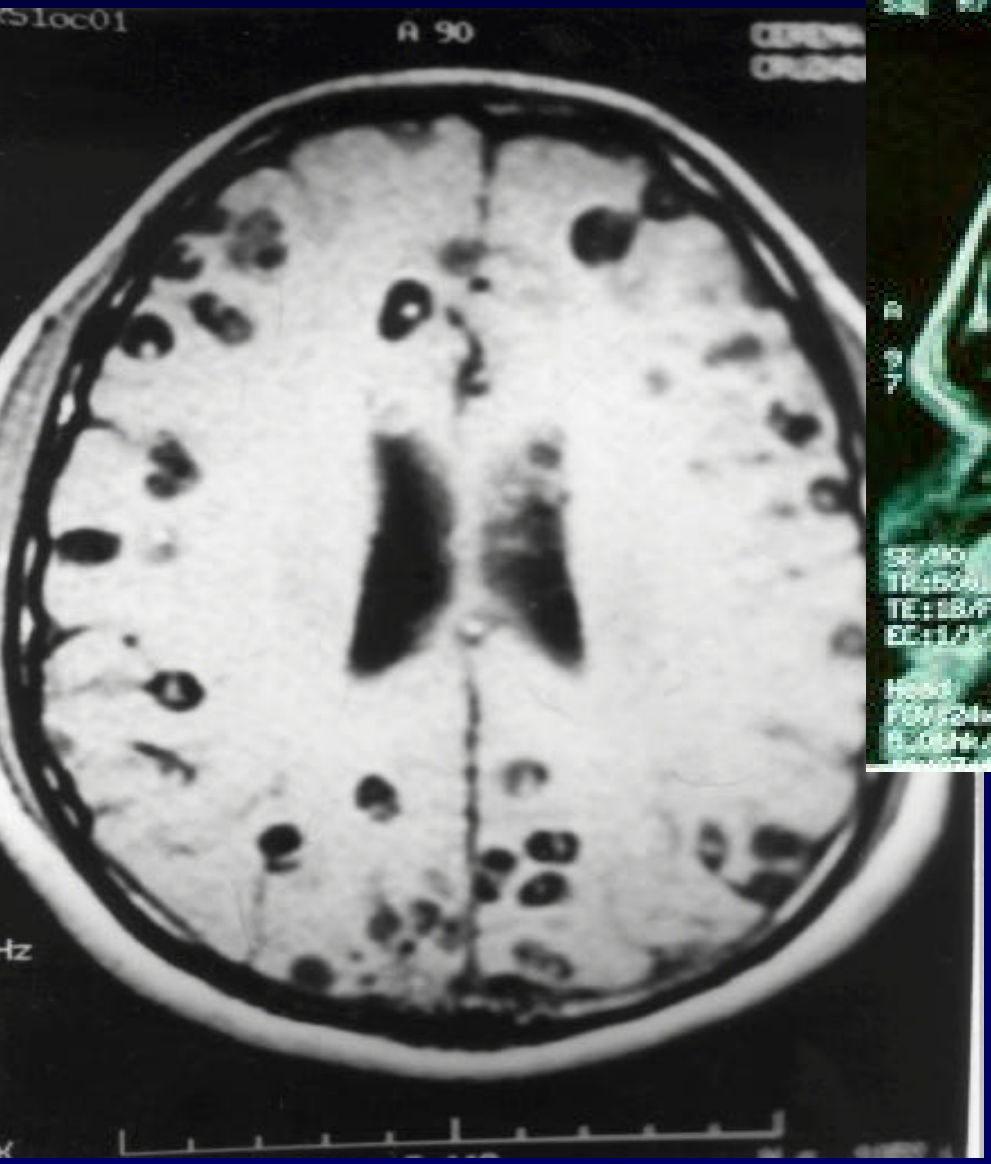
Glycolysis plus respiration

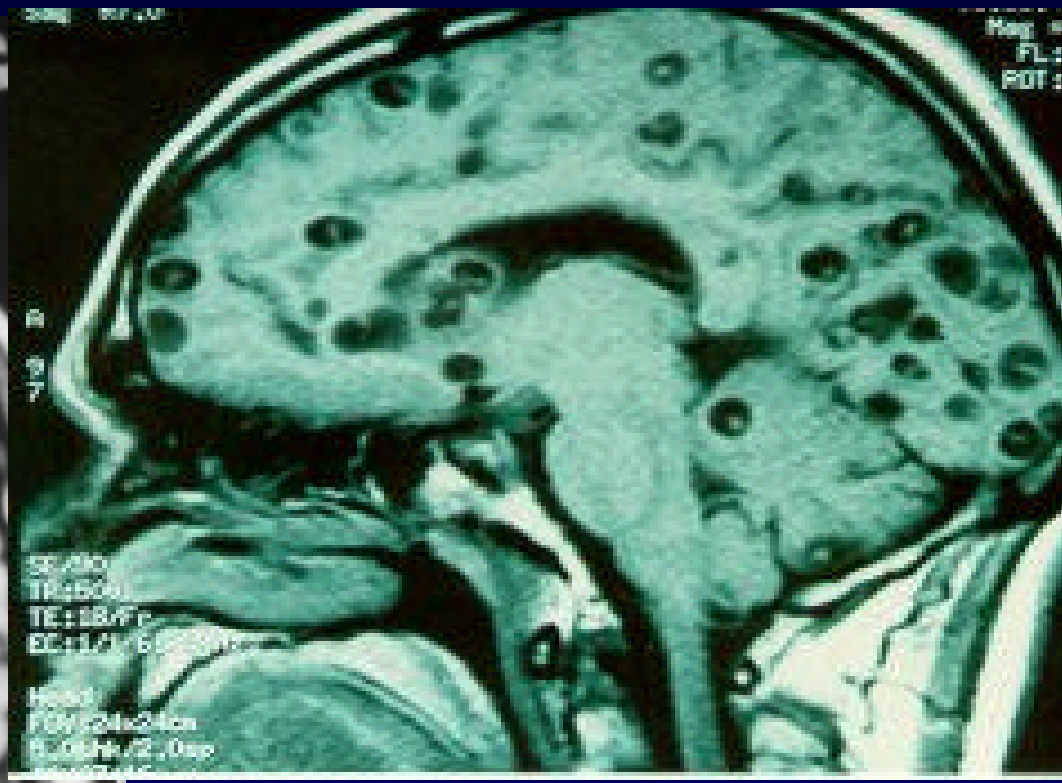
4 ATP , 10 NADH, 2 FADH2

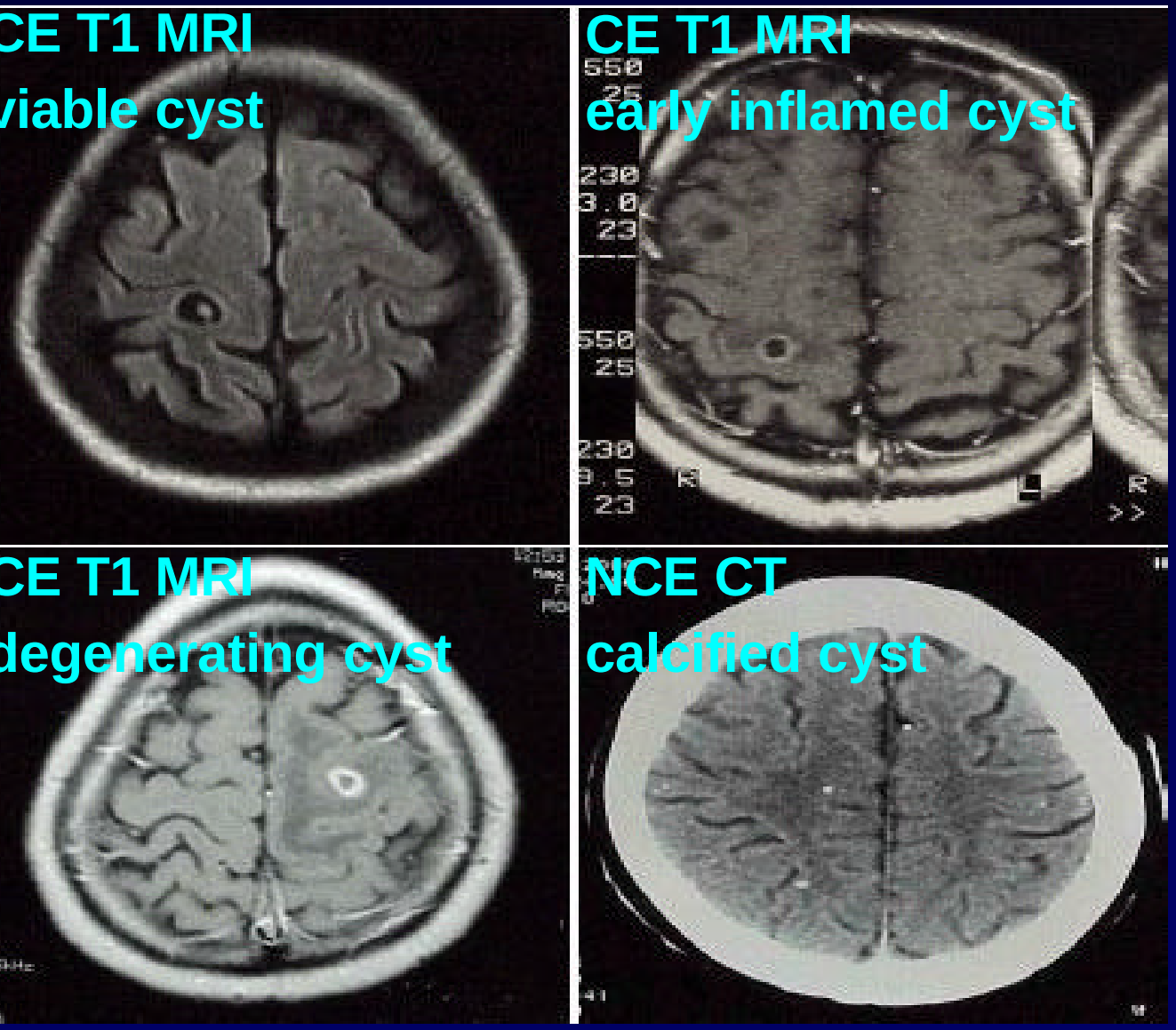
Oxidative phosphorylation can convert each NADH into 3 ATP and each FADH2 into 2 ATP. Net yield is 38 ATP, although some mitochondria use two of the ATP for transporting pyruvate.


SOMATOM PLUS
spin -40
tilt +0
M Derecho


R

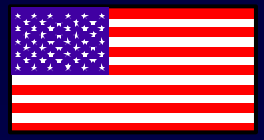

A 90





Garcia, HH et al
New Eng. J. Med.
350: 247, 2004

ynthetic antigens and quantitative immunoassa for human and porcine cysticercosis



CDC, Atlanta

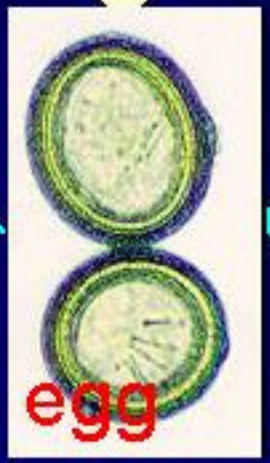
Victor Tsang
Kathy Hancock
Min Levine
John Noh
Sowmya Pattabhi
Azra Khan
Sukwan Handali
Christina Scheel
Melinda Yushak
Lynne Garrison
Holger Mayta

CWG, Peru

Robert Gilman
Hugo Garcia
Emico Gonzales
Manuela Verastequi
Silvia Rodriguez
Iskra Tuero
Yanina Arana

nding: NIH-ICIDR/TMRC, NIH/CDC-IAA, Wellcome Fd, CDC-EI, Peru OH, Gates Foundation

T. solium life cycle



What have we done:

Developed blood tests for cysts in pigs and humans, and tests for tapeworm infections in humans

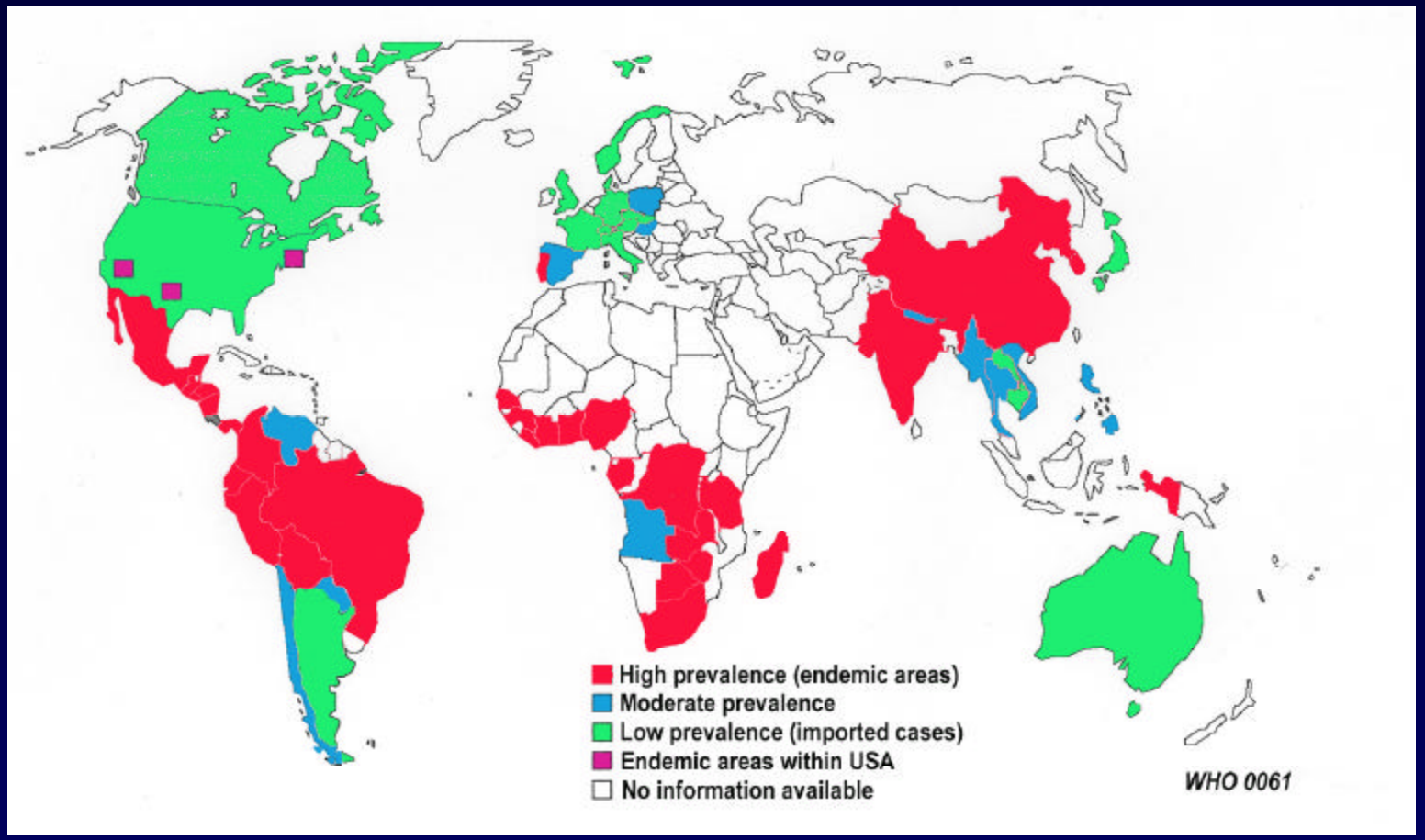
Discovered an effective and inexpensive drug, OFZ, (oxfendazole) for pigs

Discovered that OFZ-treated pigs are immune to future infection and will pass meat infection

Developed the sentinel pig model for monitoring control

Identified 2 potential vaccine molecules

Stop transmission of cysticercosis and taeniasis from 4 Peruvian villages within 6 months



- WHO: neurocysticercosis is "the most important neurological diseas
f parasitic origin in humans."
- Responsible for rates of epilepsy that are 3 - 6X higher in endemic
ountries.
- Affects ~50 million globally.

San Jan de Occollo, near Andahuaylas, Peru 1998

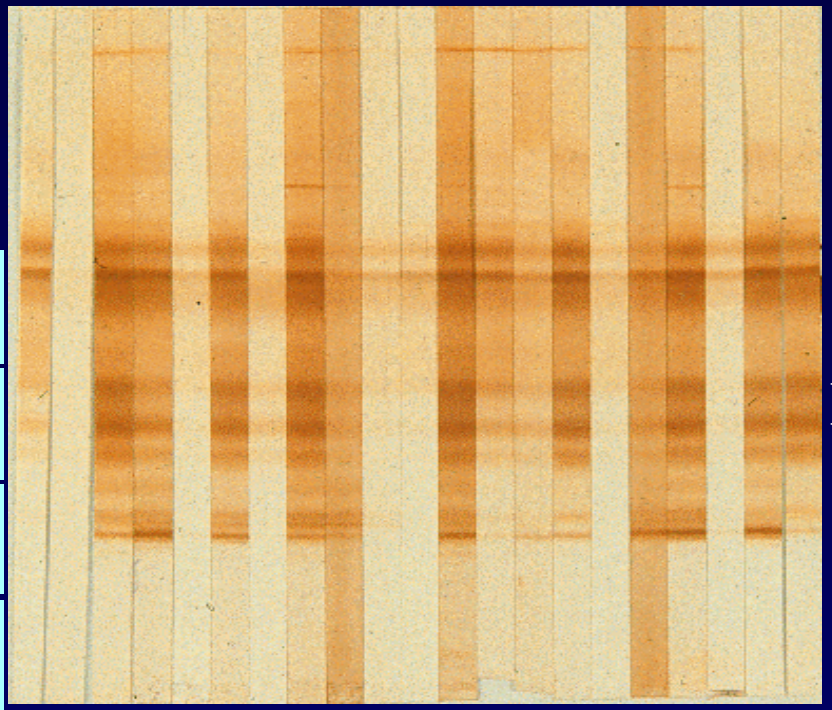
CDC western blot test for cysticercosis (EITB)

US patented (6)

Available commercially from Immunetics, Specialty Labs, MRL

WHO/PAHO preferred immunologic assay

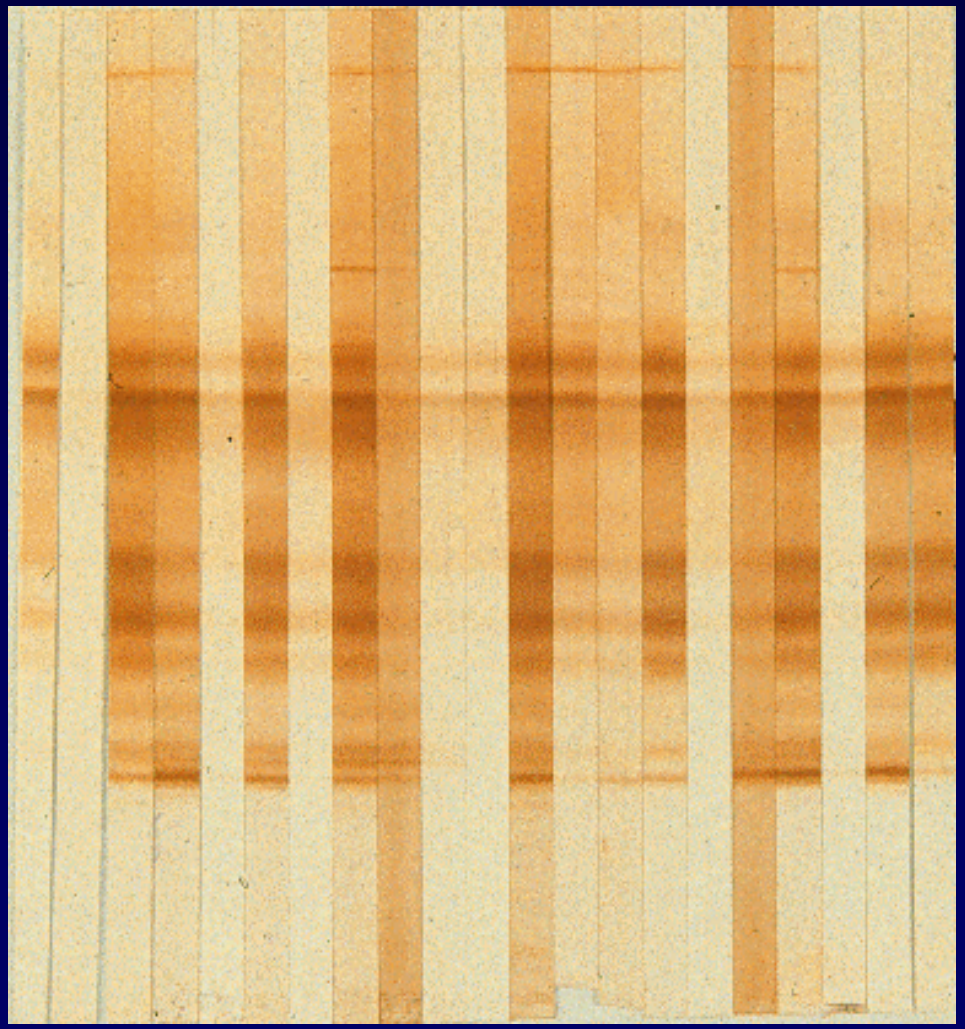
atient type	Specificity	Sensitivity
or more cysts	100%	98%
gle cyst (USA)	100%	~60%
gle cyst (Peru)	100%	~80%
gle cyst (India)	100%	~79%



Limitations of LLGP western blot

- Antigen only works in a western blot format
- A western blot is not a field assay
- Purification of LLGP requires parasite material
- Purification requires sophisticated equipment and technical expertise

CDC western blot test for cysticercosis



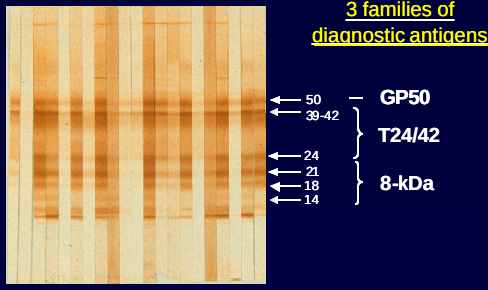
3 families of diagnostic antigens

Steps in developing recombinant antigen diagnostic assays

- Identify
- Purify
- Sequence
- Clone
- Synthesize or Express → **T24/42**
- Evaluate
- Develop Assay

} **8-kDa proteins**
GP50

8 kDa proteins



- Found at 14-, 18-, and 21-kDa in LLGP
- Components of the bands at 24- and 39- to 42-kDa
- Hydrophillic proteins
- pI = 9
- Signal sequence
- Mature proteins are 66 or 67 amino acids
- 1 to 3 cysteines
- 1 to 3 N-linked glycosylation sites

-kDa gene family

2 nucleic acid sequences

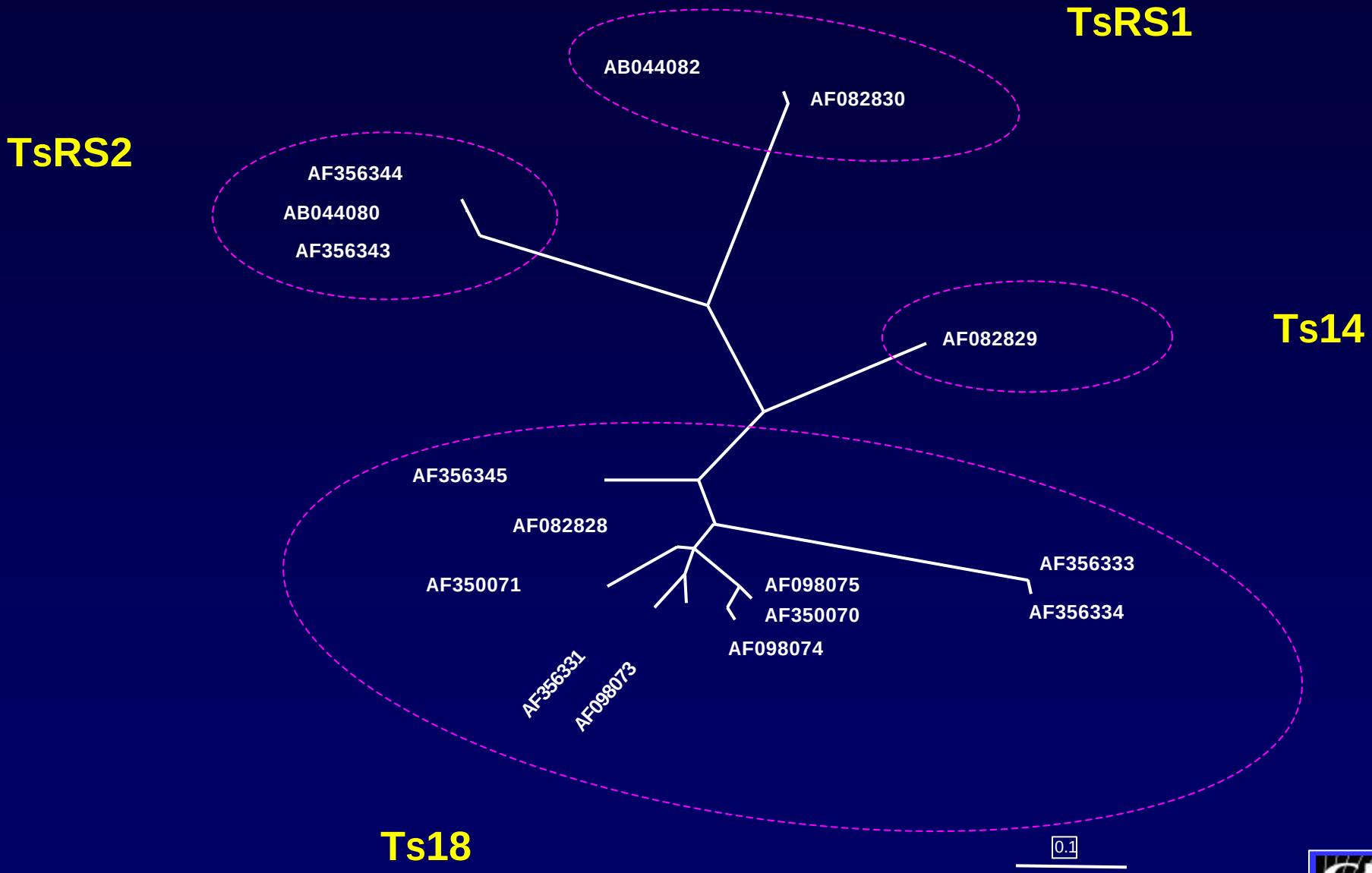
6 unique sequences

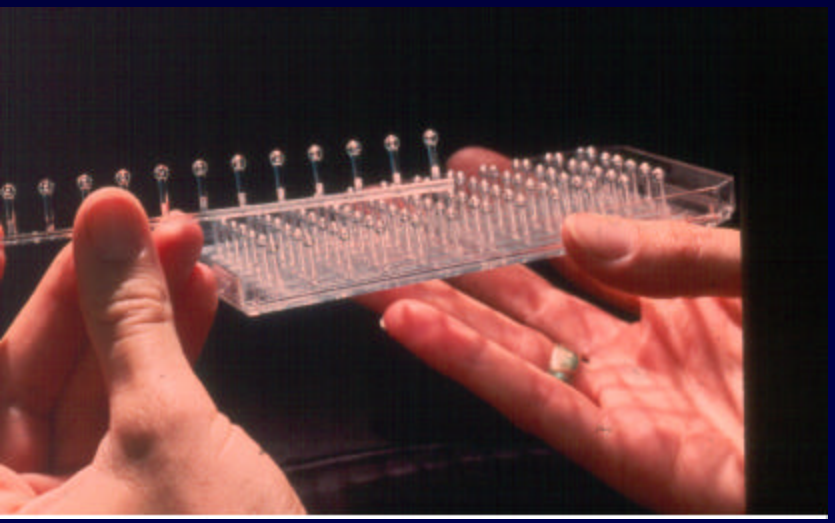
3 unique protein sequences

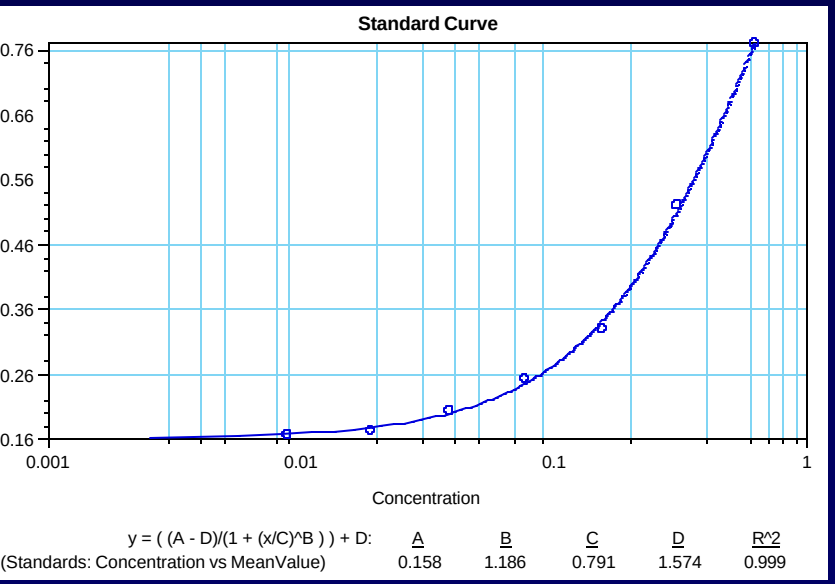
3 unique mature protein sequences

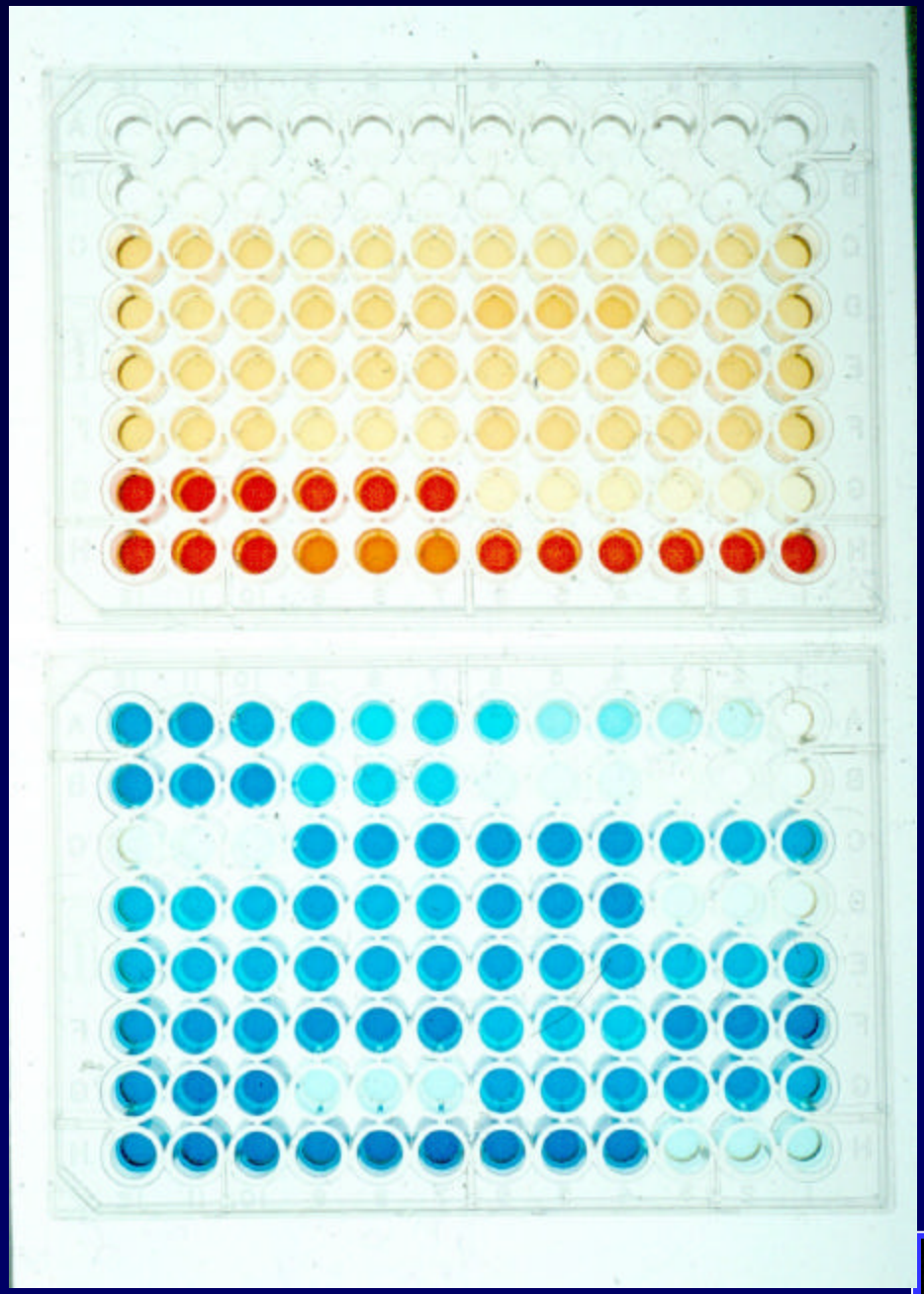
GenBank accession #	Name	Origin	Source	Reference
AF082829 AF158184 AF257776	Ts14 Ts14 Ts14	mRNA genomic mRNA	Peru Peru Mexico	Greene et al., 2000 Greene et al., 2000 Obregon-Henao et al., 2001
AF356335 AF356336	Ts14 Ts14	genomic genomic	China India	this ref. this ref.
AF356337	Ts14 var1	genomic	India	this ref.
AF356338	Ts14 var2	genomic	India	this ref.
AF356339	Ts14 var3	genomic	India	this ref.
AF098073 AF356330	Ts18 var1 Ts18 var1	mRNA genomic	Peru Peru	Greene et al., 2000 this ref.
AF098074	Ts18 var2	mRNA	Peru	Greene et al., 2000
AF098075	Ts18 var3	mRNA	Peru	Greene et al., 2000
AF356331	Ts18 var4	genomic	India	this ref.
AF356332	Ts18 var5	genomic	China	this ref.
AF350070	Ts18 variant 1	mRNA	Mexico	Obregon-Henao et al., 2001
AF350071	Ts18 variant 2	mRNA	Mexico	Enciso – direct submission
AF082828	Ts18	mRNA	Peru	Greene et al., 2000
AB044081	TSOLAg1V1	mRNA	China	Sako et al., 2000
AF356333	Ts18 var6	genomic	China	this ref.
AF356334	Ts18 var7	genomic	China	this ref.
AF356345	Ts18 var8	genomic	India	this ref.
AF356341 AF216695 AF163972	TsRS1 Ts21 immunogenic protein	genomic mRNA mRNA	Peru China China	this ref. Liu – direct submission Bin – direct submission
AB044083	TSOLAg2V1	mRNA	China	Sako et al., 2000
AB044082	TSOLAg2	mRNA	China	Sako et al., 2000
AF082830	TsRS1	mRNA	Peru	Greene et al., 2000
AF356340	TsRS1 var1	genomic	India	this ref.
AF356342	TsRS1 var2	genomic	China	this ref.

Phylogenetic tree of *T. solium* 8-kDa proteins



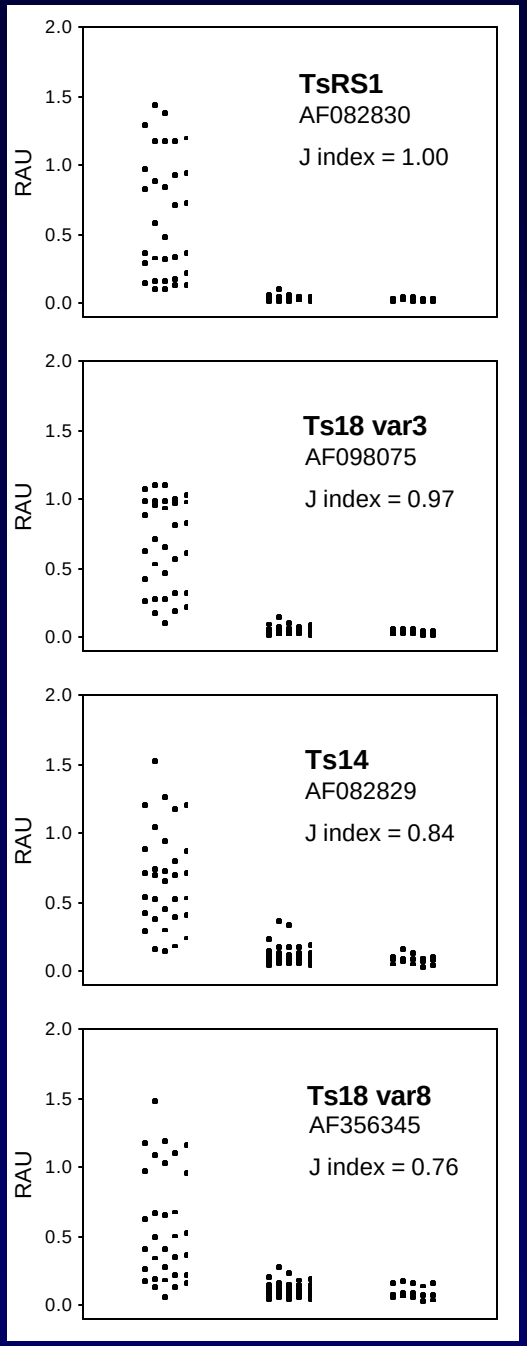


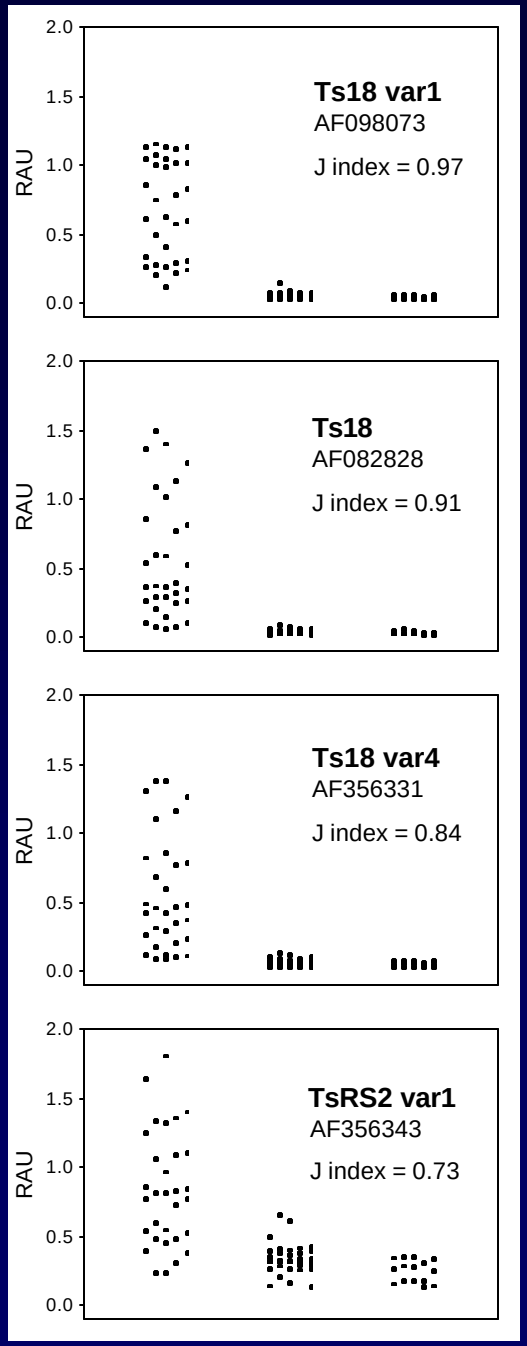

Standard Curve
0.76
0.66
0.56
0.46
0.36
0.26
0.16
0.001
0.01
0.1
1
Concentration
y = ((A - D)/(1 + (x/C)^B)) + D:
(Standards: Concentration vs MeanValue)
A
0.158
B
1.186
C
0.791
D
1.574
R^2
0.999



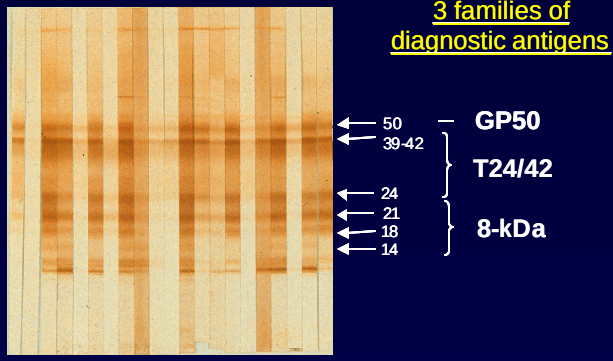
Antibody reactivity

8-kDa proteins





GP50 sequence



signal peptidase cleavage site

ALTAVLIFVVSTSSENAPKMWGSRVIGKPSGPSDTMSYEYNDNYRTVLINDSVLGTMSIKRNQCMLWETKPW
CNIFPGYVNITLNNVTAQKIMEMDEITARPRVASTTFFVPHCNFTKPAPGEVDVWTSFPLSRFVKDTPWFRVD
GGANYDSTATFDINATSLCFWRGTKLLHKGAEFCTDMVKDESADLRVFRGVFPRKTNISRESFAFAGLKTALT
YSQSGISPEVADCKQYAKVKDLSTLVATMPAYATKTSTGNNSKTTSSGPASTNAFKAIIALLLIPMVL

GPI anchor attachment site

Mature protein

260 amino acids = 28.9 kDa

7 N-linked glycosylation sites

6 cysteines

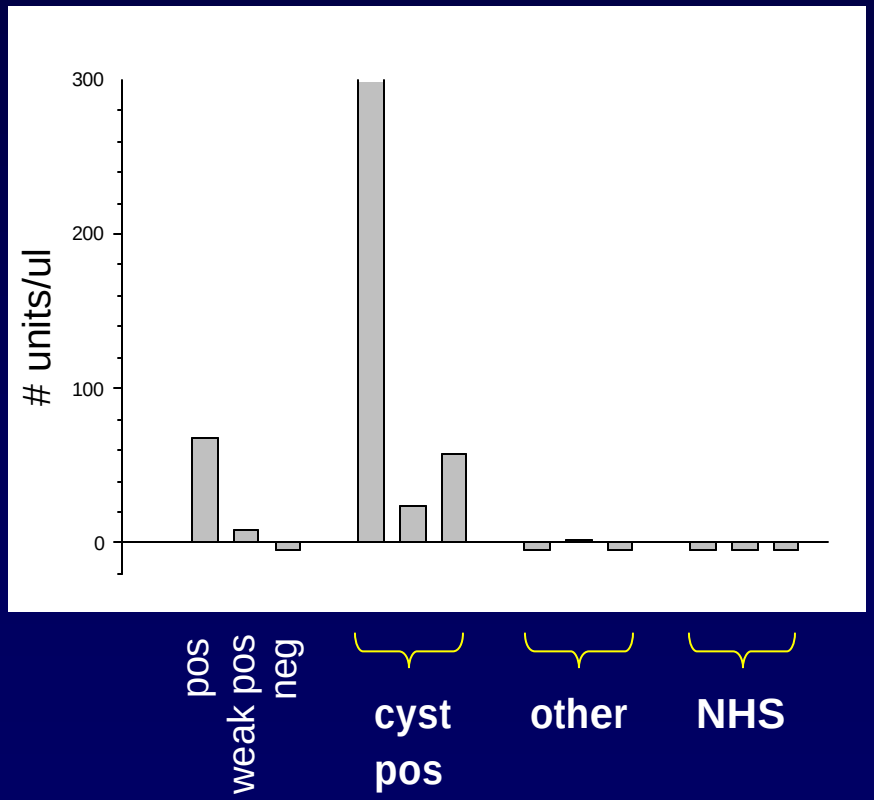
Lateral flow with rGP50

trol →
est →

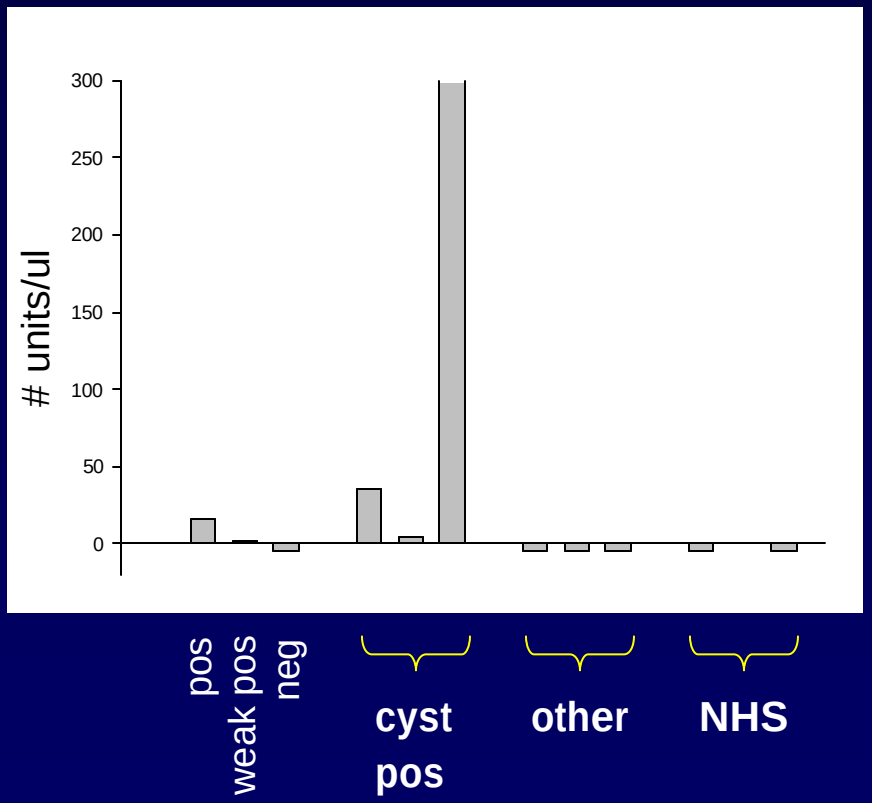
units/ul 109 2 2 114 11 13 91 50

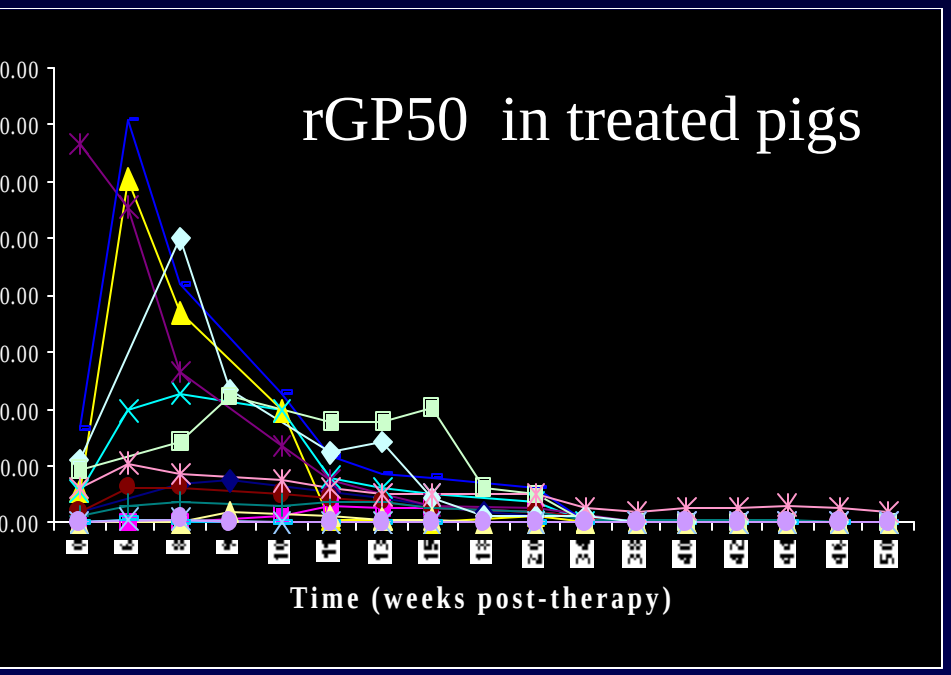
InBios ELISA

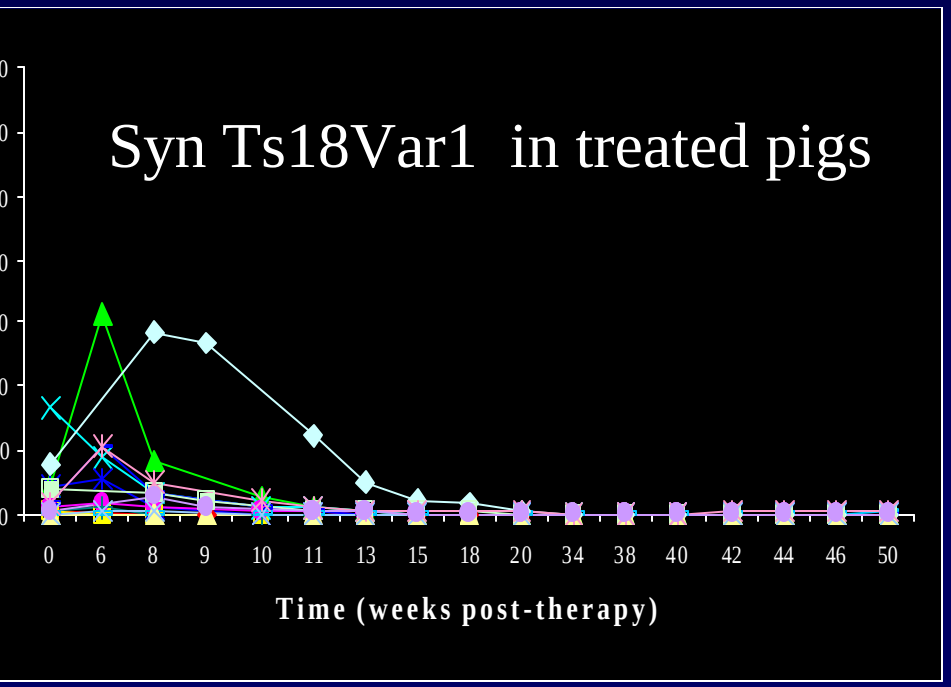
sTs14



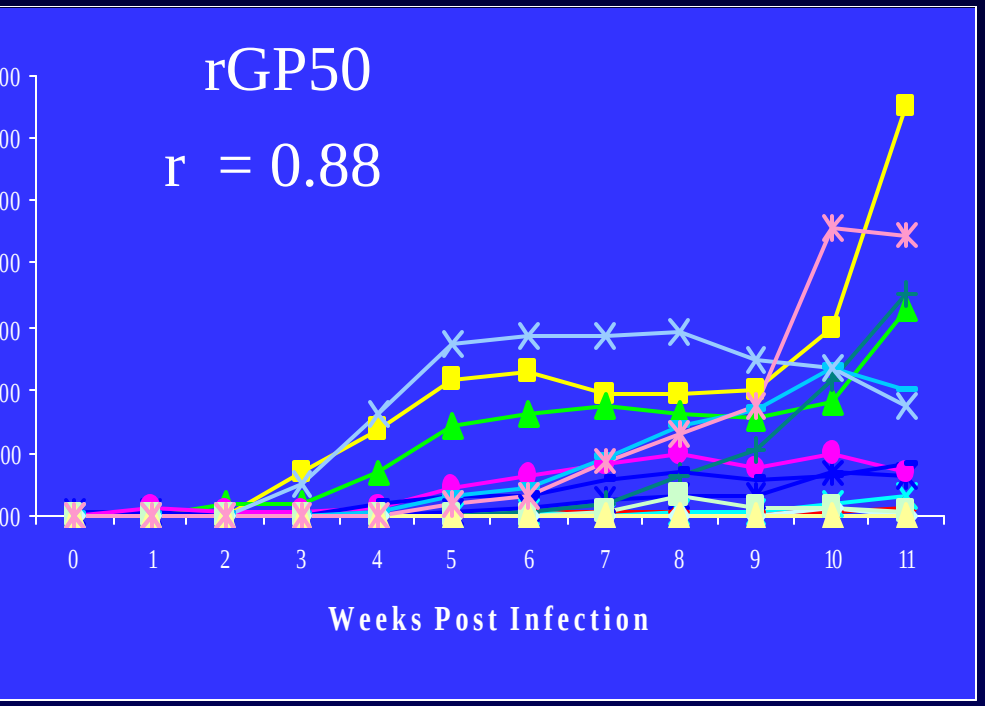
rGP50

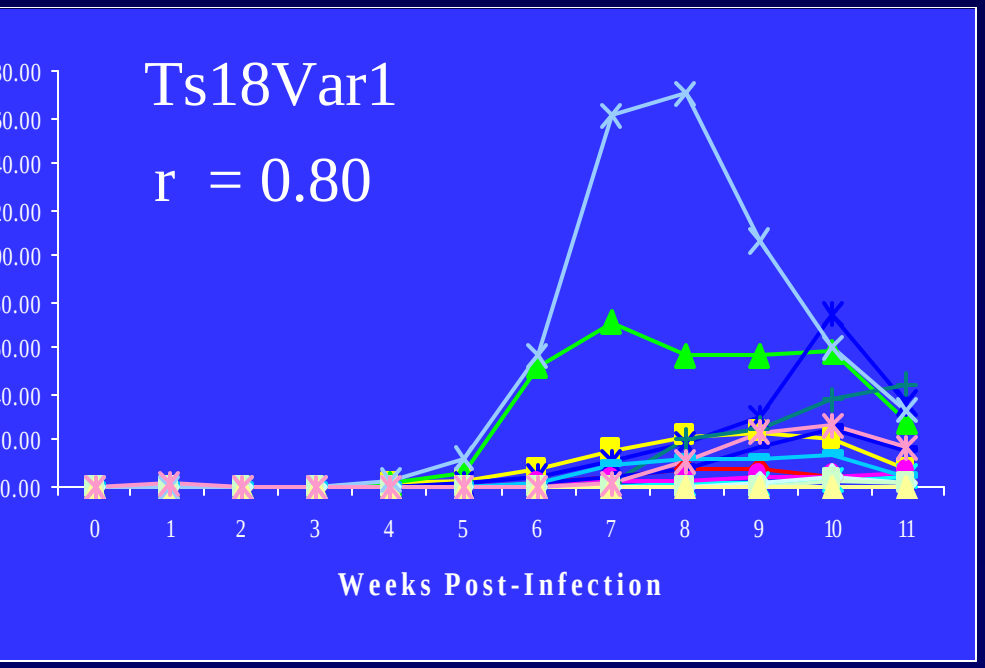






- Antibodies to synthetic antigens can be used to monitor treatment success in pigs.
- Antibodies to 8 kd antigens (Ts18Var1) diminish faster than those to rGP50.





[Antibody] can be correlated with number of viable cys in experimentally infected pigs.

Summary

- 2 synthetic/recombinant antigens for diagnosis of cysticercosis (8-kDa and GP50), 3rd is in the works (T24).

- 2 recombinant antigens for diagnosis of taeniasis (TSES33 and TSES38).

- Development of ELISA and lateral flow assays is underway.

Taeniasis NHS Cysticercosis

*

Western blots with sera from persons with either *T. solium* taeniasis or cysticercosis

Steps in developing recombinant antigen diagnostic assays

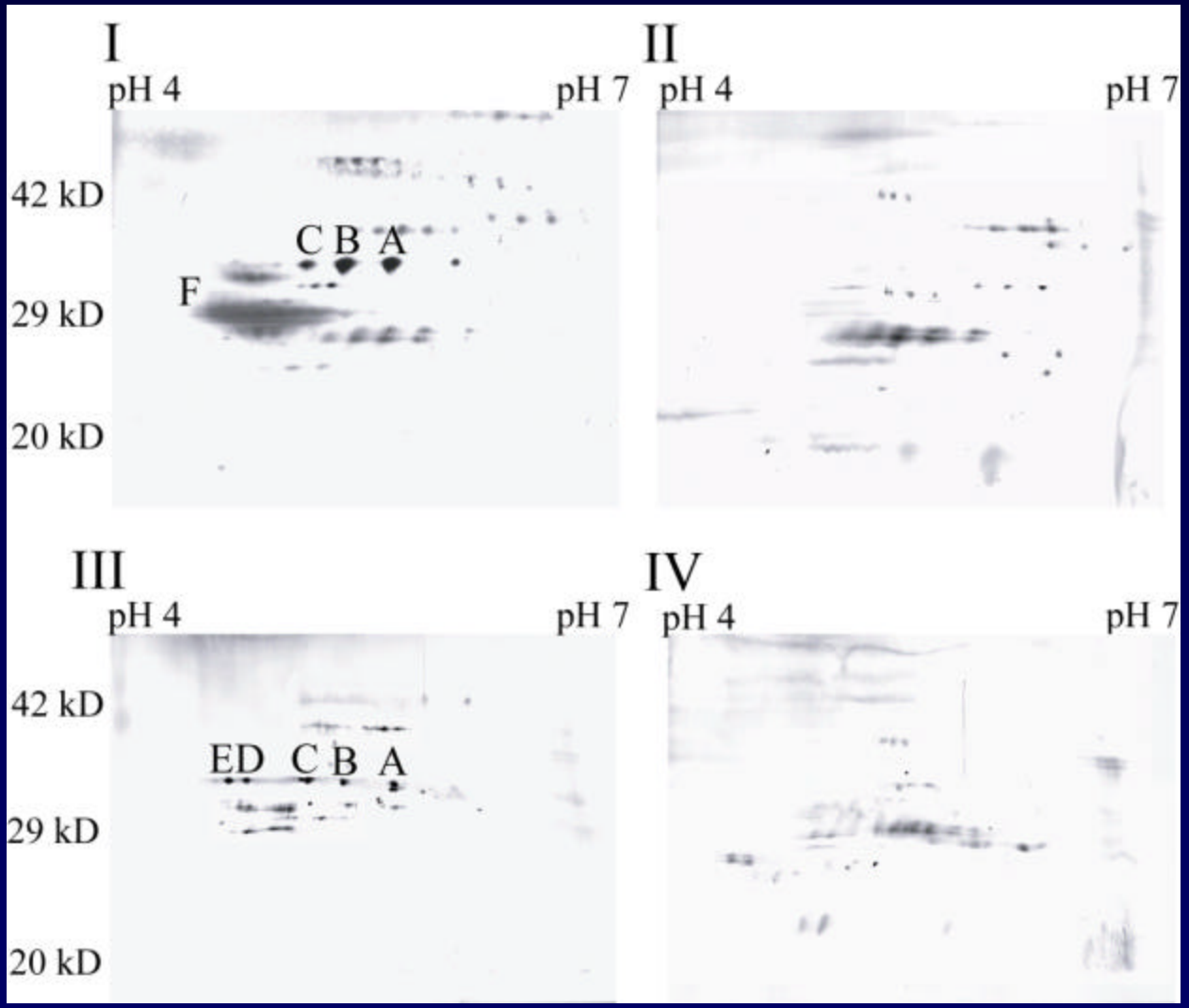
- Identify
- Purify
- Sequence
- Clone
- Synthesize or Express
- Evaluate
- Develop Assay

} **TSES33 & TSES38**

2D gels TS_{adult} ES proteins

ES proteins batch 1

ES proteins batch 2



taeniasis pos

cysticercosis pos

Survey of neurologic patients for cysticercosis (EITB)

Hospital location	% positive	Tot. survey
Beijing, PRC (seizure)	*44*	*198*
Beijing, PRC (all neurol. adm.)	*8*	*500*
Bombay, India (seizure + ?)	*32*	*107*
Rwanda (epileptic)	*21*	*34*
Mexico (epileptic)	*11*	*271*
Peru (epileptic)	*19*	*578*

Patient Enrollment and Neurocysticercosis Patients at 11 Study Sites

Site	*Total enrolled*	*No. of neurocysticercosis*
Albuquerque, NM	107	6 (5.6%)
Atlanta, GA	146	0 (0%)
Charlotte, NC	300	4 (1.3%)
Kansas City, MO	164	1 (0.6%)
Los Angeles, CA	91	9 (9.9%)
New Orleans, LA	174	2 (1.1%)
New York, NY	184	1 (0.5%)
Orlando, FL	68	0 (0%)
Philadelphia, PA	185	1 (0.5%)
Phoenix, AZ	243	10 (4.1%)
Portland, OR	171	4 (2.3%)
	1833	38 (2.1%)

Demographic and Clinical Data for Neurocysticercosis and Other ED Patients

	Neurocysticercosis n = 37	Other n = 1,796	Odds ratio 95% C.I.
edian age (years)	32 [25 - 44]	40 [30-52]	
ex male	27 (71%)	1189 (66%)	
acial/ethnic background			
lack	4 (10.5%)	746 (41.6%)	
/hite, non-Hispanic	3 (7.9%)	640 (35.7%)	
ispanic	29 (76.3%)	291 (16.2%)	16.7 [7.8 – 35.6]
nsurance status			
edicare/Private	7 (18.4%)	455 (25.3%)	
edicaid	3 (7.9%)	386 (21.5%)	
ninsured	22 (57.9%)	738 (41.1%)	2.5 [1.2 – 5.3]
mmigrant status			
orn in the US	5 (21%)	815 (62%)	
ot born in the US	12 (50%)	166 (13%)	11.8 [4.1 – 33.9]
nknown	7 (29%)	343 (26%)	
xposure to endemic region			
o travel out of the US	0 (0%)	950 (52.9%)	
xposure to endemic region	28 (73.7%)	314 (17.5%)	172 [11 - 2830]
nknown travel history	9 (23.7%)	532 (29.6%)	
rior history of neurocysticercosis			
ositive prior history	3 (16%)	5 (0.5%)	34.0 [7.5 – 154]
o prior history	16 (84%)	906 (99.5%)	

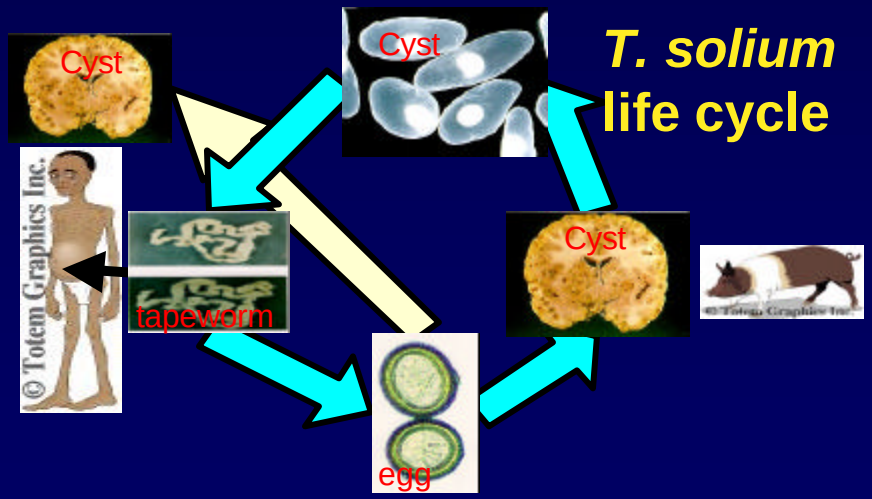
Risk factors associated with EITB seropositivity for cysticercosis in 946 Peruvian neurologic patients

Risk Factors	No of Cases	No. EITB+	% EITB+	p
Pig raising**	421	108	26	<0.001
Born outside of Lima, Peru**	622	149	24	<0.001
Older than 20 years**	660	143	22	<0.001
Seizures**	504	112	22	<0.001
Dirt floor	97	29	30	<0.005
History of taeniasis**	108	29	27	<0.05
House in rural area	100	27	27	<0.05
No sewage in house	149	37	25	<0.05
Fewer than 3 bedrooms	401	84	21	<0.05
Residency out of Lima	269	48	18	<0.05

Antibodies to *T. solium* cystic antigens in relationship to pig raising habits, among general villagers of Saylla, Peru. Population = 501

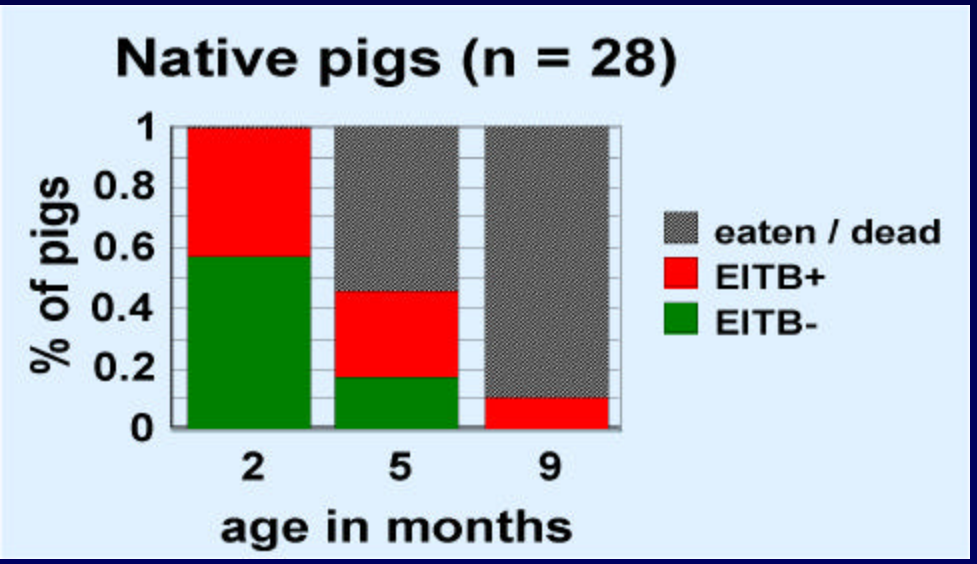
	n	EITB +	Prevalence %	Odds ratio [95%C
Do not raise pigs	32	3	9.4	1.0
Raise pigs	69	21	30.4	4.2 [1.1-23.8]
Also butcher their pigs	60/69	20	33.0	4.8 [1.2-27.4]
Also sell pork	47/60	19	40.4	6.6 [1.6-37.6]
Also sell chicharrones	14/47	7	50.0	9.7 [1.6-68.7]

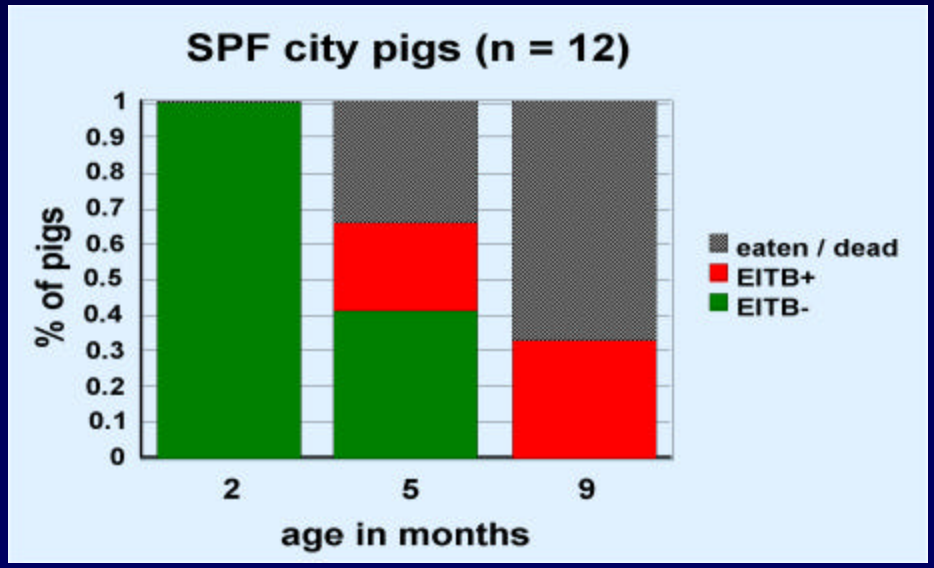
Can we use the good diagnostic tests to develop other tools for controlling these diseases?



EITB and Sentinel pigs

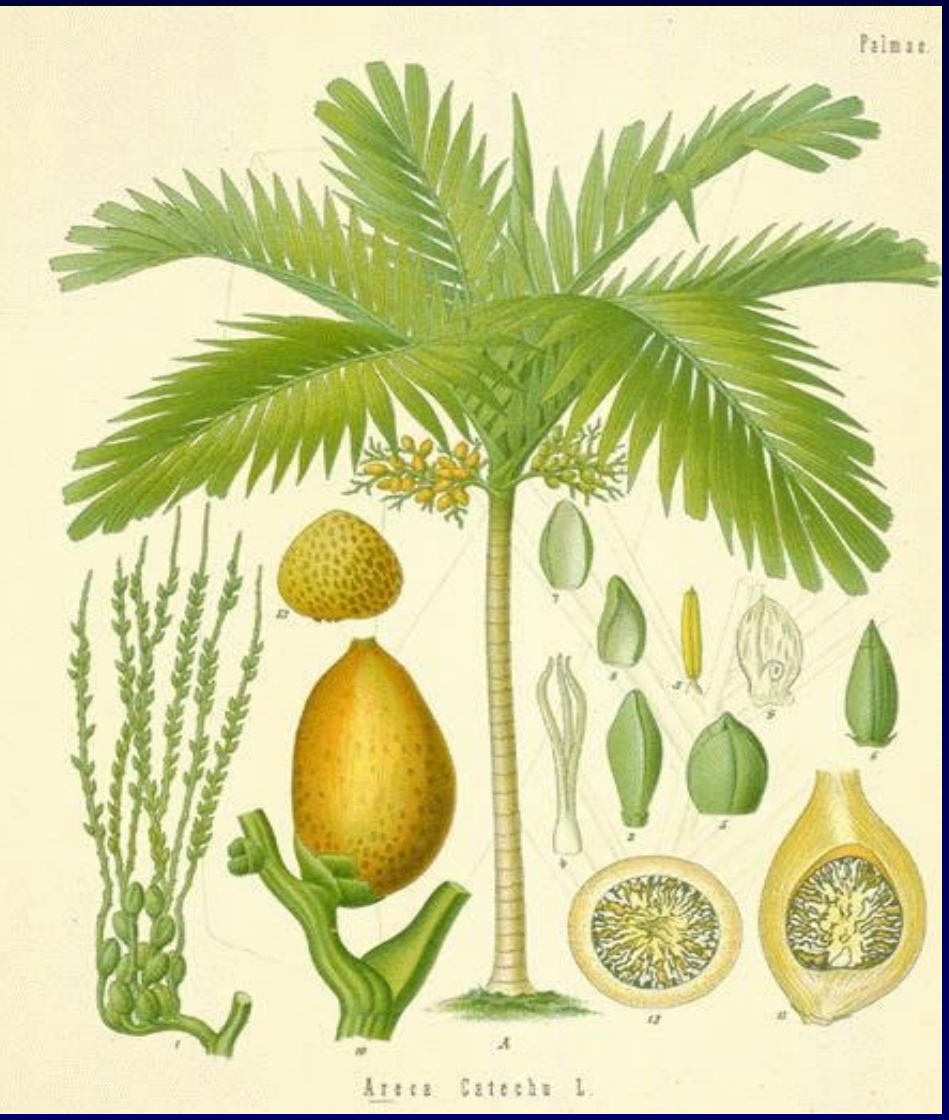
are effective tools for monitoring environmental levels of infective eggs



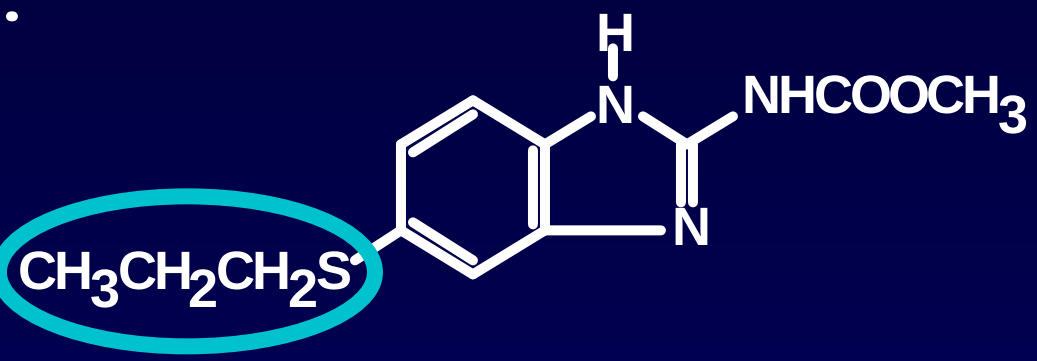



©W.P. Armstrong 2000

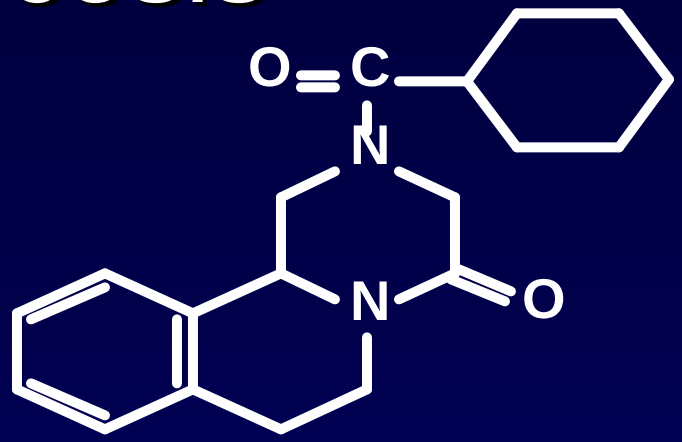

PUMPKIN


Palmae.
Areca Catechu L.

New drug for cysticercosis



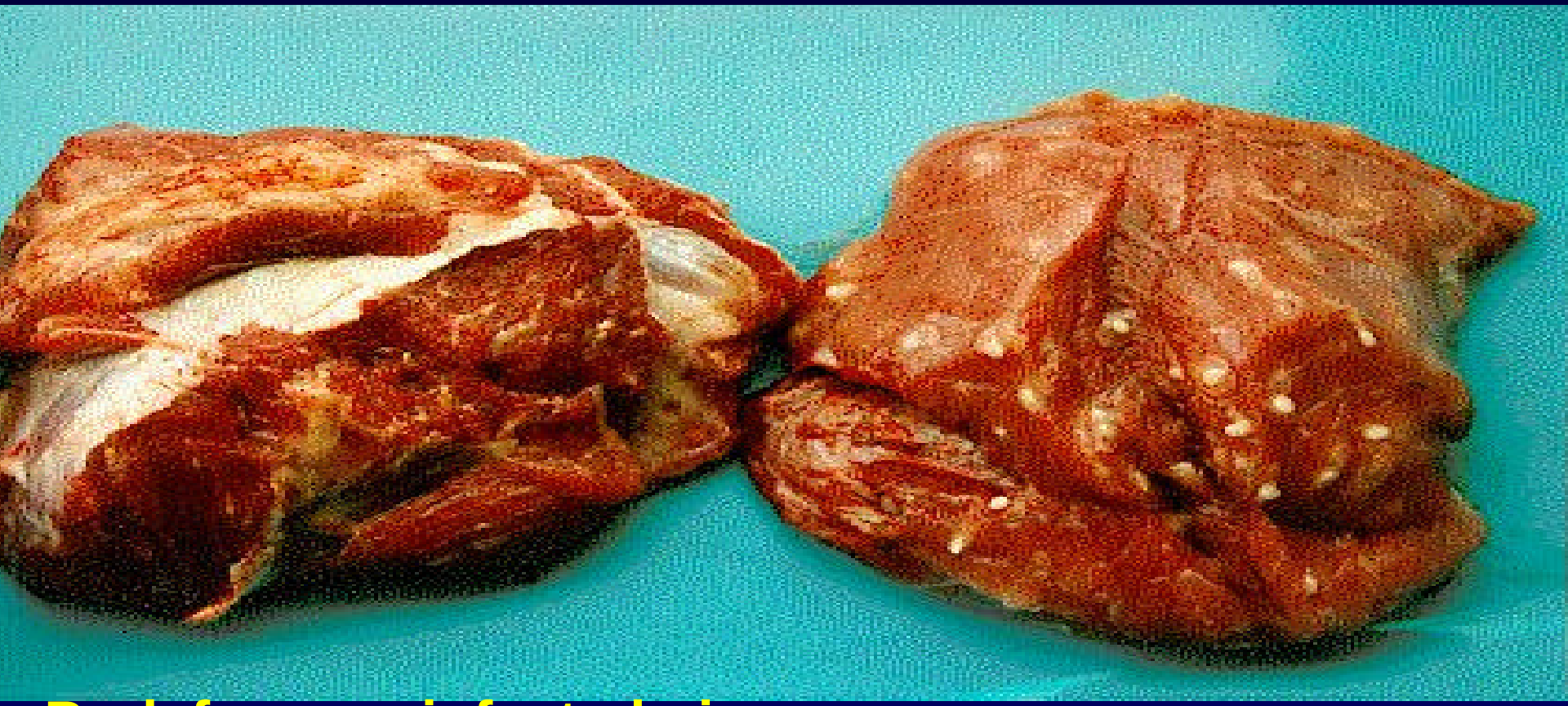
Albendazole $10.00*



Praziquantel $40.00*

Oxfendazole $1.00*

*cost (US$) to treat 1 average 60kg pig



Pork from an infected pig treated with a single dose (30 mg/kg) of Oxfendazole

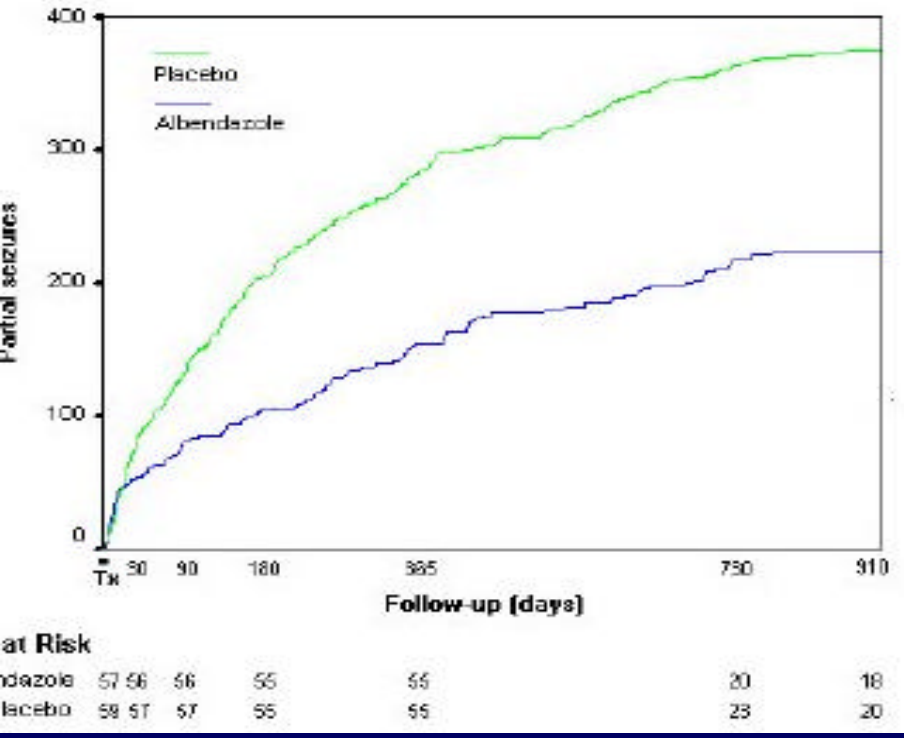
Untreated

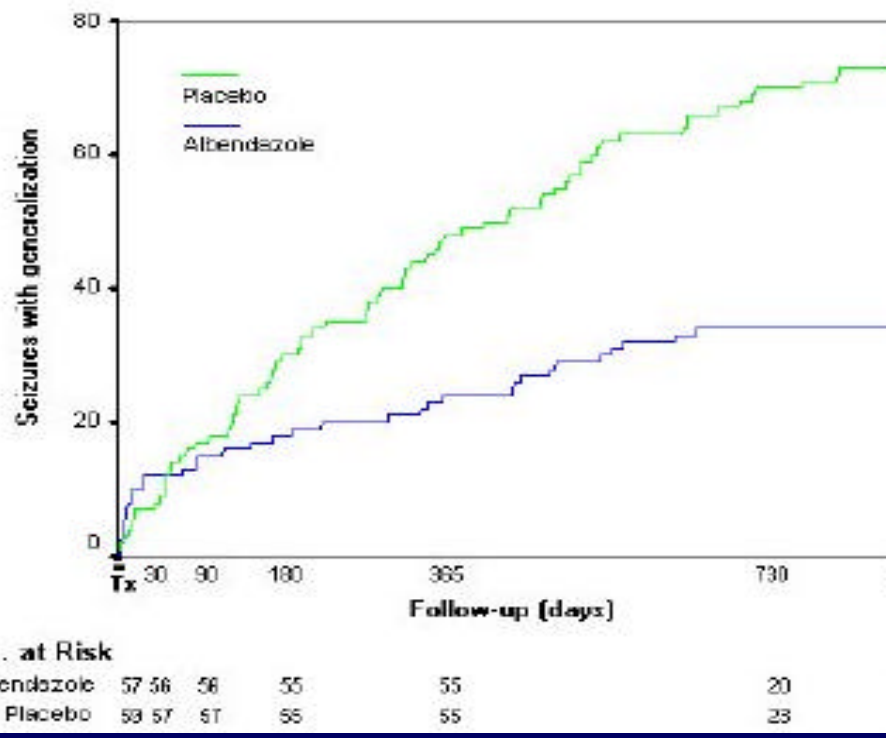
T. solium cysts found at necropsy in the carcasses of OFZ-treated pigs and naive controls 3 months after exposure (for a period of 3 months) to infection.

Findings at necropsy*	OFZ-treated pigs (n=19)	Naive controls pigs (n=32)
Viable cysts only	0	3
Viable and degenerated	0	4
Degenerated cysts only	0	5
Residual scars only	15	0
No cysts	4	20

no viable cysts were found in the carcasses of any of the 19 treated pig
12/32 versus 0/19, p=0.001, Fisher's exact test)

Anti-parasitic Rx is advantageous to the patient





Garcia, HH et al, New Eng. J. Med.,350: 247, 2004

TSES33 & TSES38 sequences

TSES33

Spots A, B, and C

N-terminal signal sequence

251 amino acids

Predicted mol wt - 27.5 kDa

Predicted pI – 5.81

glycoprotein

6 cysteines

TSES8

Spots D and E

N-terminal signal sequence

262 amino acids

Predicted mot wt – 28.8 kDa

Predicted pI – 4.68

glycoprotein

6 cysteines

Neurocysticercosis Patients at 11 U.S. Emergency Department Study Sites

Site	*Total seizure patients enrolled*	*Neurocysticercosis patients identified*
Albuquerque, NM	107	6 (5.6%)
Atlanta, GA	146	0 (0%)
Charlotte, NC	300	4 (1.3%)
Kansas City, MO	164	1 (0.6%)
Los Angeles, CA	91	9 (9.9%)
New Orleans, LA	174	2 (1.1%)
New York, NY	184	1 (0.5%)
Orlando, FL	68	0 (0%)
Philadelphia, PA	185	1 (0.5%)
Phoenix, AZ	243	10 (4.1%)
Portland, OR	171	4 (2.3%)
	1833	38 (2.1%)

Control strategy

- ★Survey pigs and humans
- ★Treat all pigs with Oxfendazole
- ★Test and treat tapeworm carriers
- ★Test sentinel pigs
- ★Latrine & water
- ★Education

Demonstration Elimination program:

140 villages, ~100,000 population

EITB+: Human = 8-18%, pig = 25-80%

<u>Control strategy comparison:</u>

1 Tx of human taeniasis

2 Pig OFZ Tx of pigs

3 Sentinel pigs (EITB) for monitoring outcome

4 Pig vaccination

funding: NIH-ICIDR, CDC-EI, Peru MOH/MO, & Gates Foundation

Indication of intervention efficacy as measured by percent incidence of new infections in naive native pigs.

INTERVENTION VILLAGES	Nov 99 / Febr 00	Mar 00 / May 00	Jun 00 / Aug 00	Sept 00 / Dec 00	Jan 01 / Apr 01	May 01 / Jul 01	Aug 01 / Nov 01	Dec 01 / Mar 02
Isla Noblecilla	46.2	10.0	11.1	14.3	14.3	**0**	4.8	**0**
Leandro Campos	2.2	**0**	**0**	3.3	9.7	3.5	3.8	2.7
Matapalo	4.1	4.2	8.6	7.4	17.4	7.1	1.0	13.2
Nuevo Progreso	9.9	5.4	8.5	24.2	14.0	21.3	16.3	15.6
Quebrada Seca	6.7	9.4	**0**	14.6	6.3	15.0	4.2	19.7
Totora	3.8	**0**	8.7	7.1	9.5	7.1	17.6	20.5
Tutumo	1.7	1.9	2.2	2.7	9.7	5.1	4.7	6.3

- Intervention was a combination of mass pig treatment, and taeniasis treatment of humans. Post intervention incidences of pigs return to ~12%.
- Arrows show interventions performed.
- Zero incidences (obtained in four villages , in six periods) indicate no new infections, or eliminatio of transmission.

Program for cysticercosis: field - lab integrated and systematic

- Produce efficacious tests
- Develop effective patient management
- Determine prevalence
- Develop effective drugs
- Develop effective & sustainable prevention/control strategy
- Develop vaccine for pigs
- Develop local partnership for control programs
- **Final goal = Elimination / eradication**

ding: NIH-ICIDR/TMRC, NIH/CDC-IAA, CDC-EI, Wellcome Fd, Peru MOH, Gates Foundation