

3rd International Symposium on Alternatives to Antibiotics (ATA)

**NOVEL VACCINE ANTIGENS IDENTIFIED BY
CHICKIEN MONOCLONAL ANTIBODIES
AGAINST APICOMPLEXANS**

**M. Matsubayashi¹, I. Teramoto-Kimata², S.Uni³,
H.S. Lillehoj⁴, & K. Sasai^{1*}**

- 1. Graduate School of Life and Environmental Sciences, Osaka Prefecture University.**
- 2. Graduate School of Medicine, Osaka City University.**
- 3. Department of Public Health, Faculty of Nursing, Kobe Women's University.**
- 4. Animal Biosciences and Biotechnology Laboratory, Beltsville Agricultural Research Service, Agricultural Research Service, U.S. Department of Agriculture.**

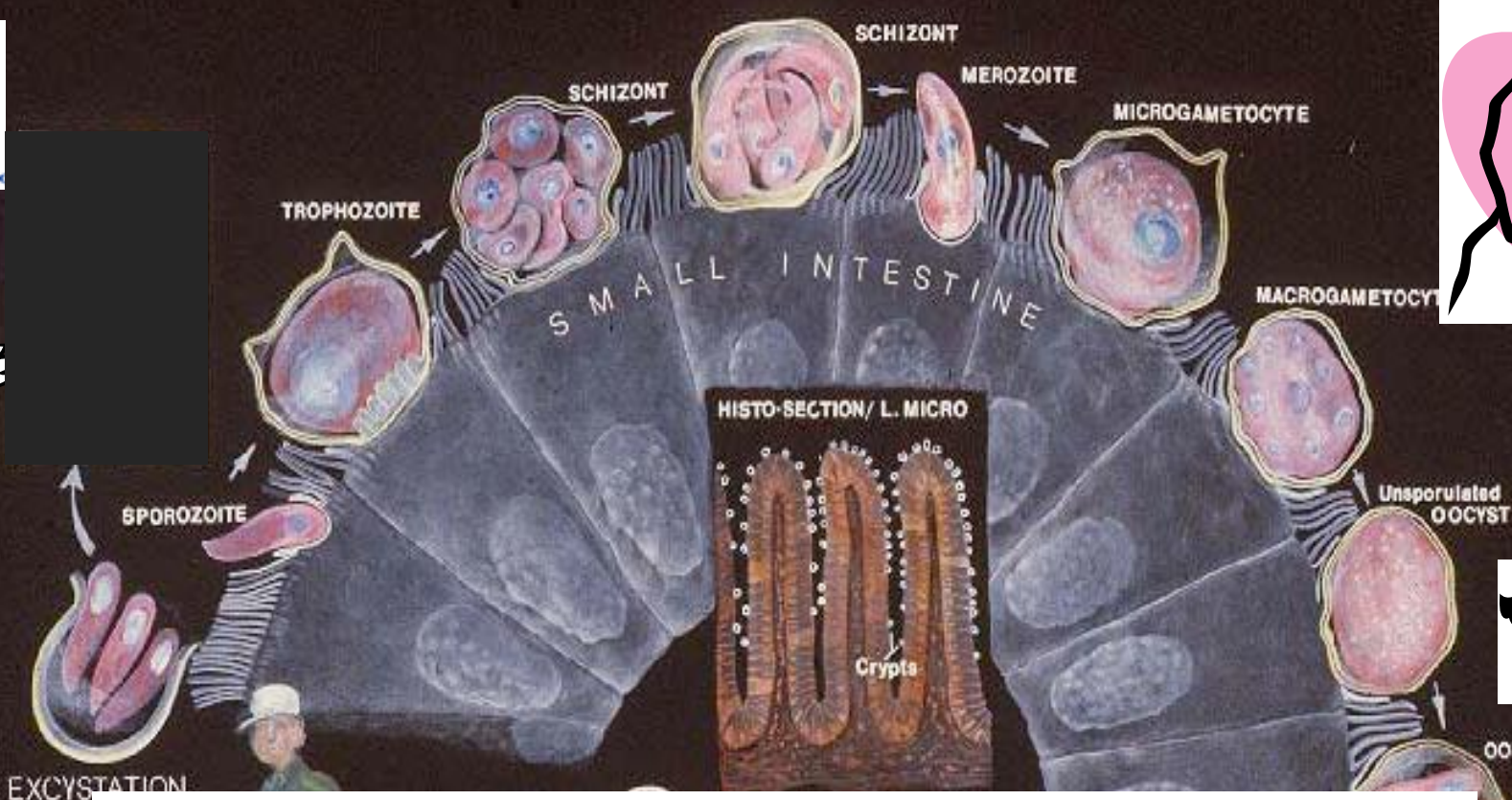
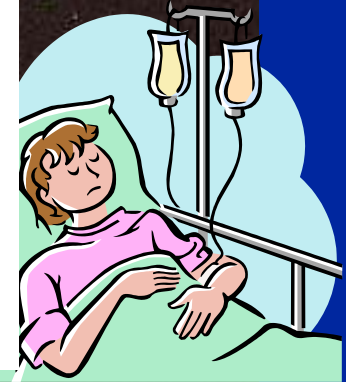
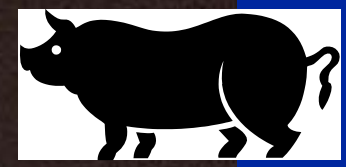
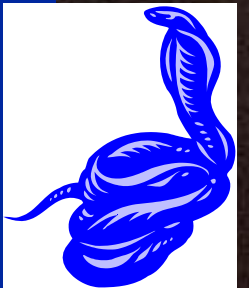


Apicomplexan parasites

- ◆ *Toxoplasma*
- ◆ *Cryptosporidium*
- ◆ *Malaria*
- ◆ *Neospora*
- ◆ *Babesia*
- ◆ *Eimeria*

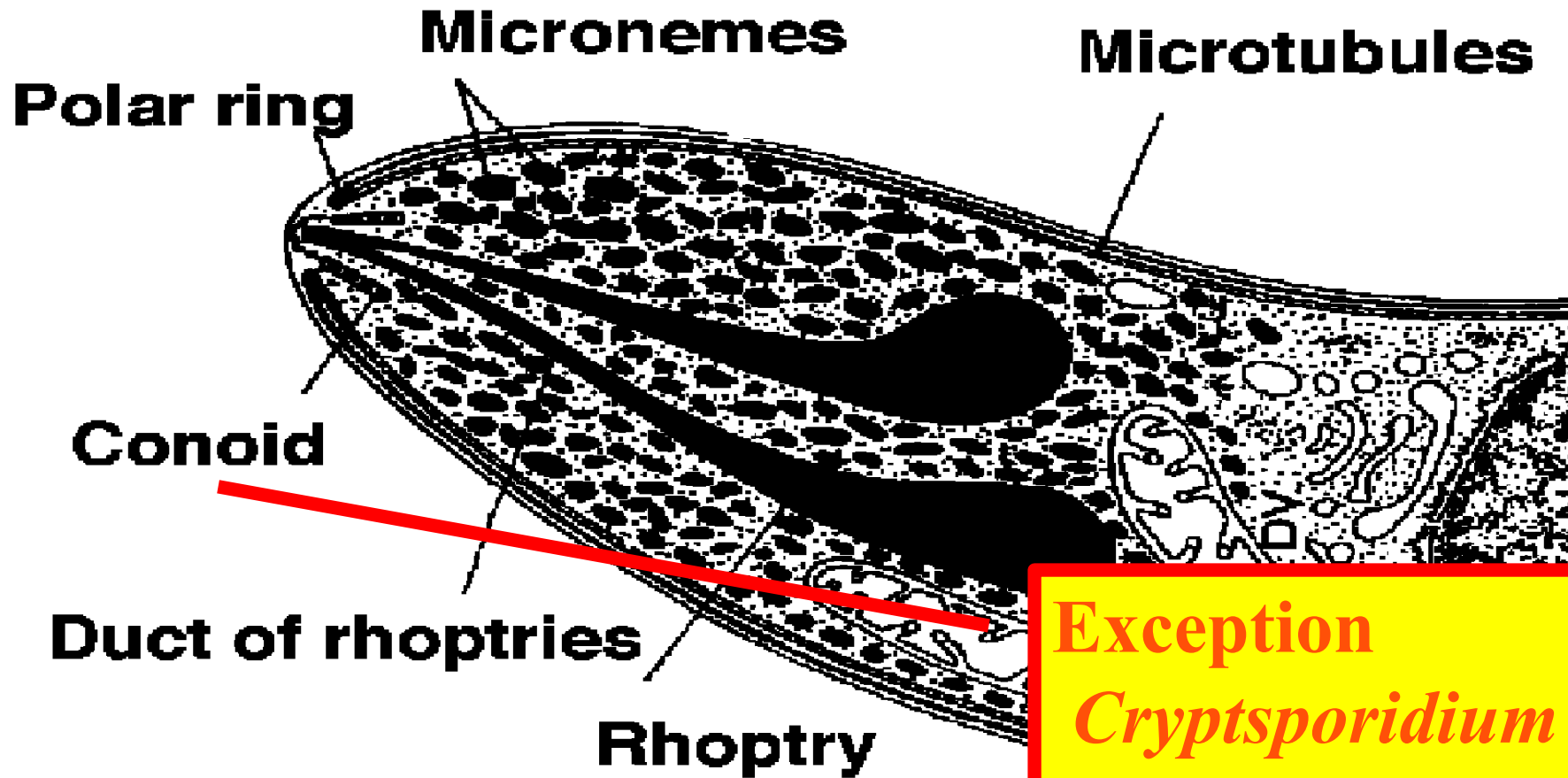
**Many of
them were
Zoonosis**

**Many of them were
big problems for
human, live stocks
& poultry industry**



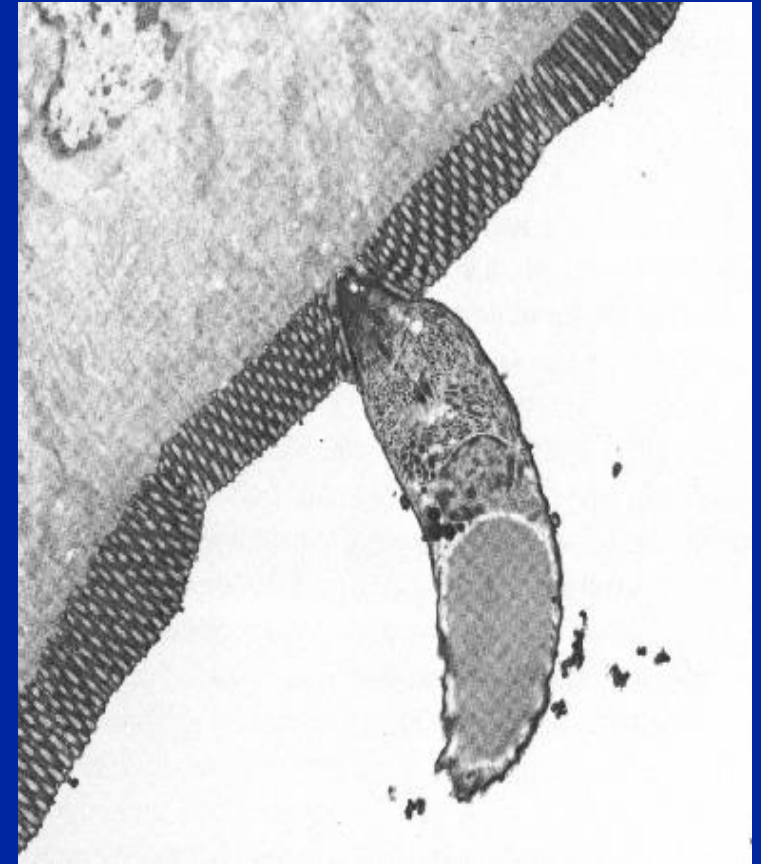
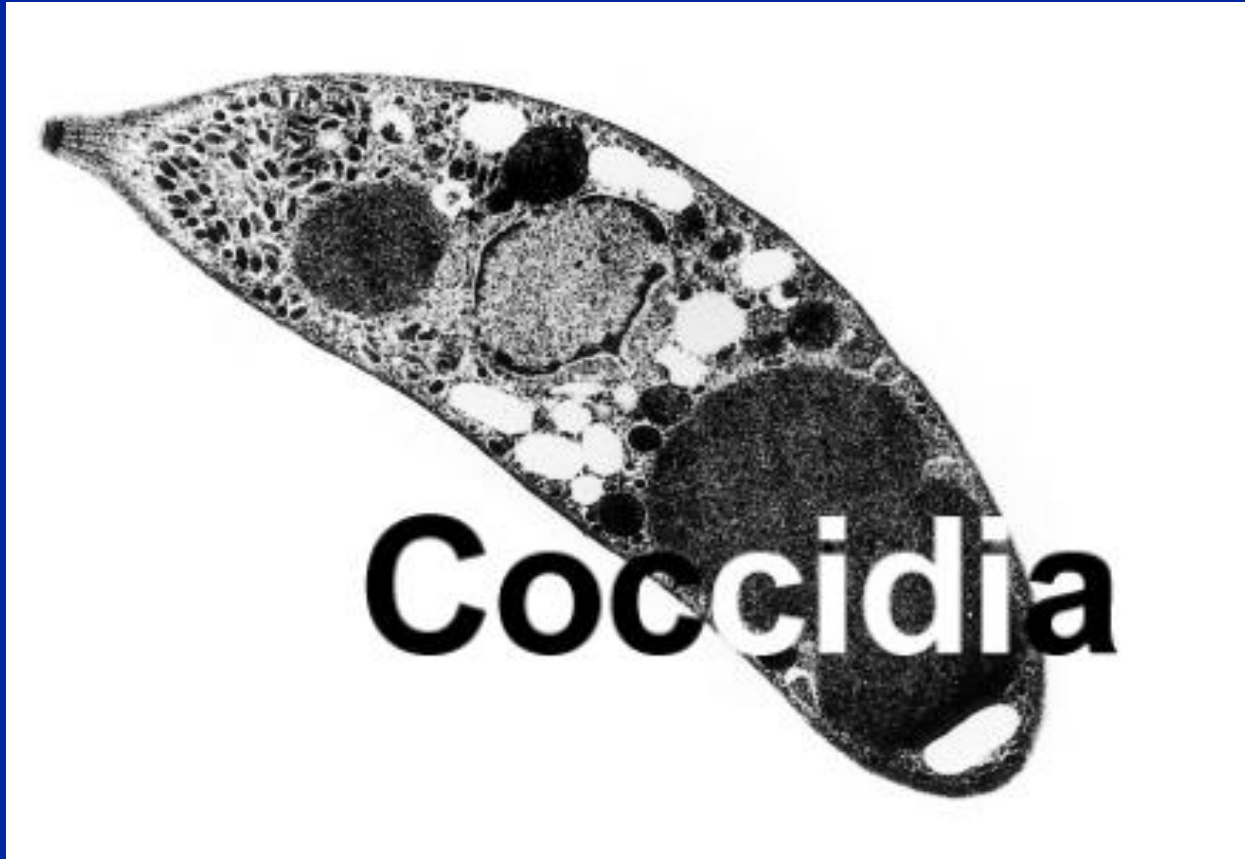
All hosts make gametogony

Ultrastructural apical complex of Aicomplexan parasite



Scholtyseck, E. 1979

Ultrastructural apical complex of Aicomplexan parasite



The invasion process of Apicomplexan parasite

A series of complicated steps

⊙ **Host cell recognition**

⊙ **Parasite movements**

⊙ **Cell to cell adhesion**

⊙ **Regulated protein secretion**

⊙ **Conoid penetration**

⊙ **Induction of a parasitophorous vacuole**

⊙ **Interiorization of the parasite**

PARASITE

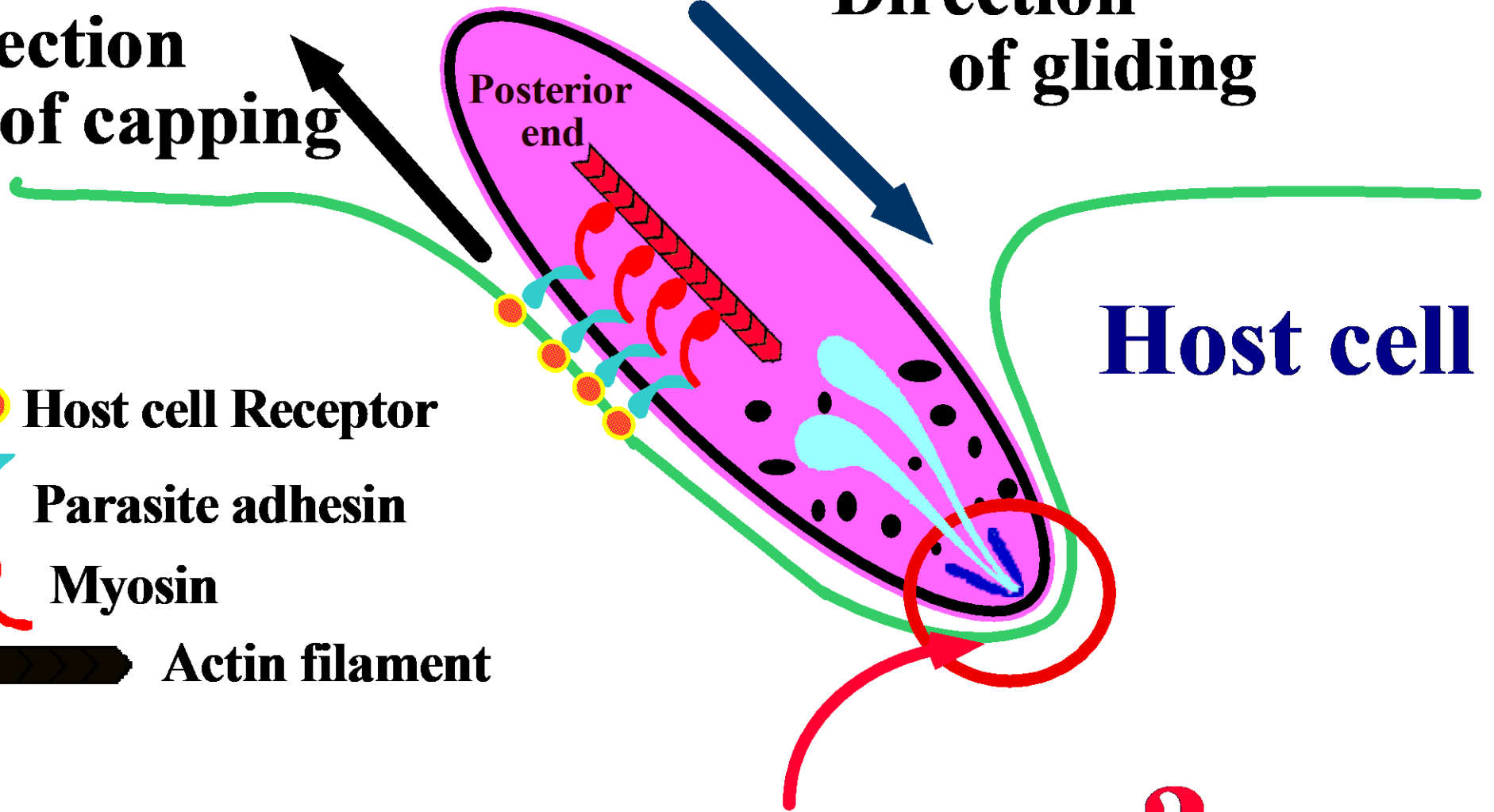
Direction of capping

Direction of gliding

Host cell

- Host cell Receptor
- ☞ Parasite adhesin
- ☞ Myosin
- ▬ Actin filament

Conoid penetration ?

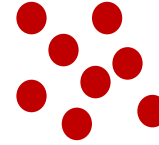


Chicken monoclonal antibody

Gastrointestinal
parasites
parasitizing
chickens
(*Eimeria*)



B Lymphocytes



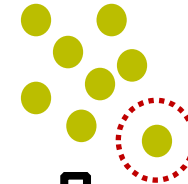
+



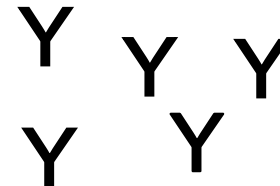
Chick myeloma

Cell fusion

Screening

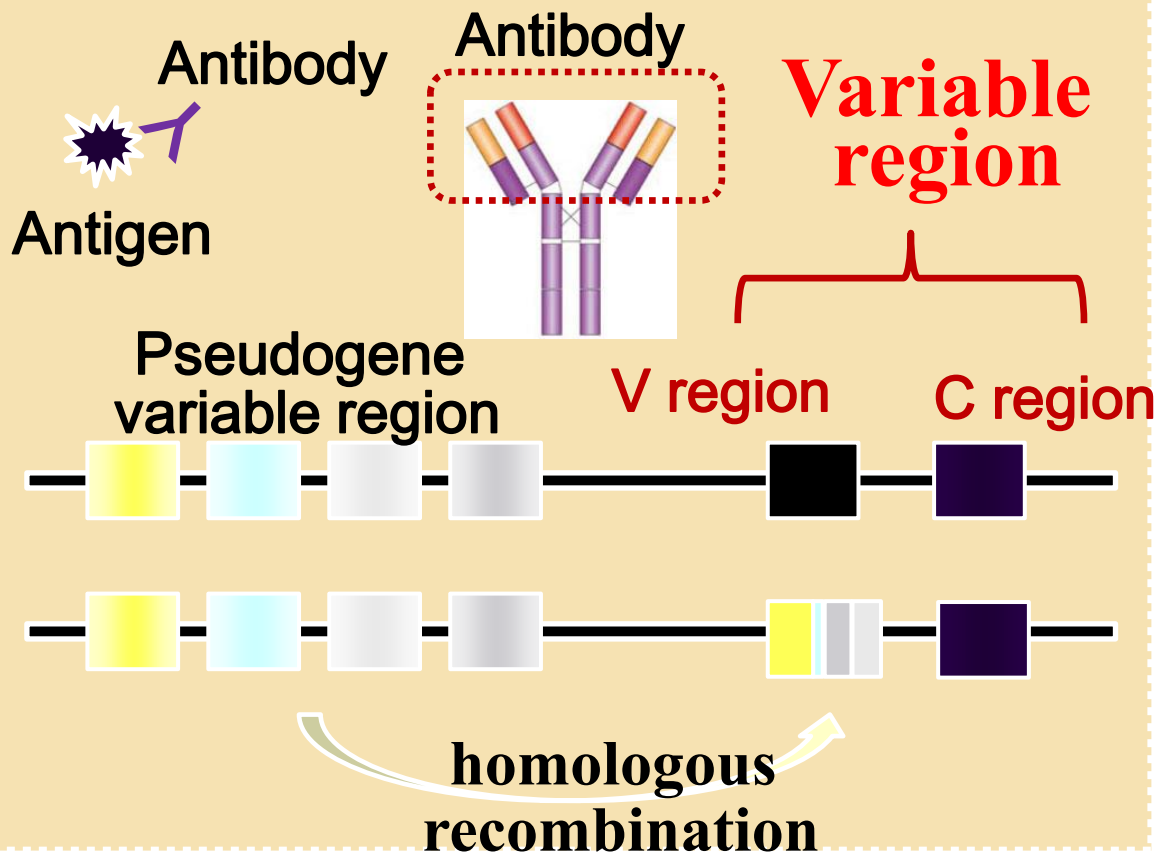


Cloning



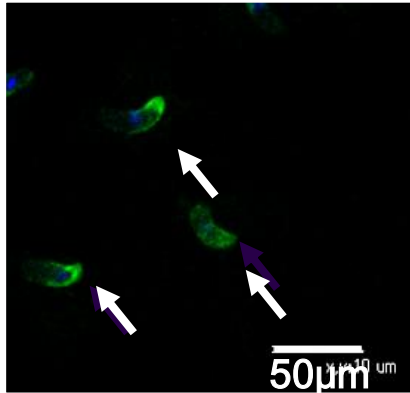
Chicken Monoclonal
Abs

(Sasai et al., J. Parasitol. 1996他)

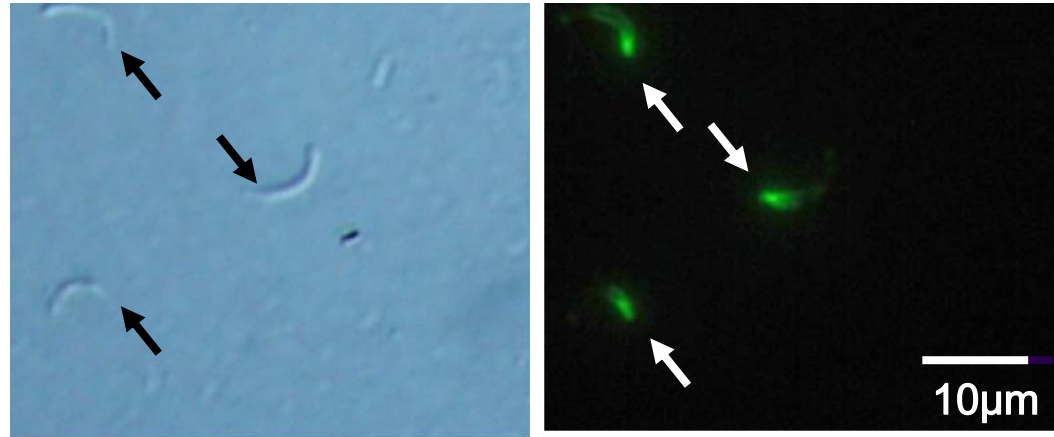


Characteristics of chicken monoclonal antibody (mAb)

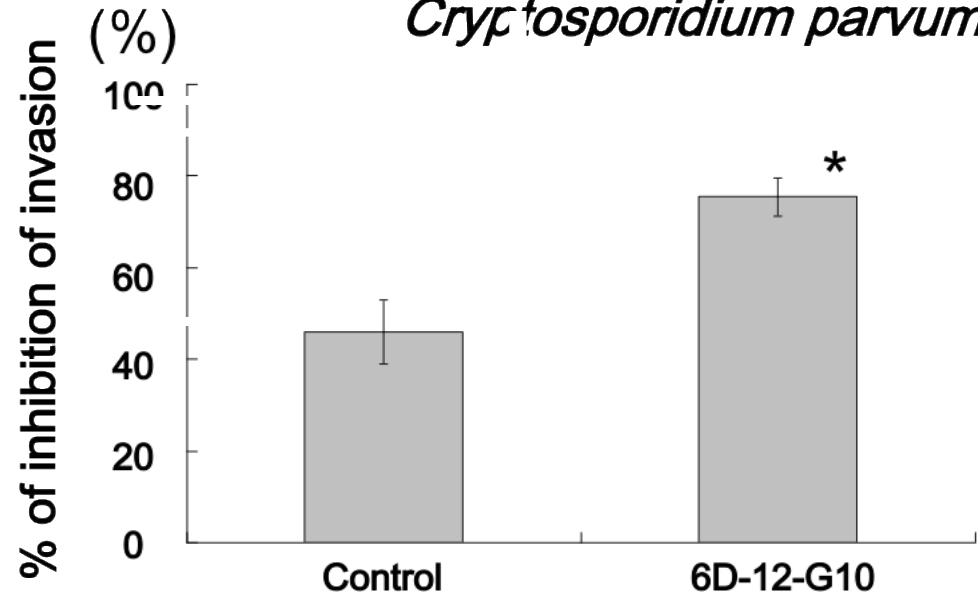
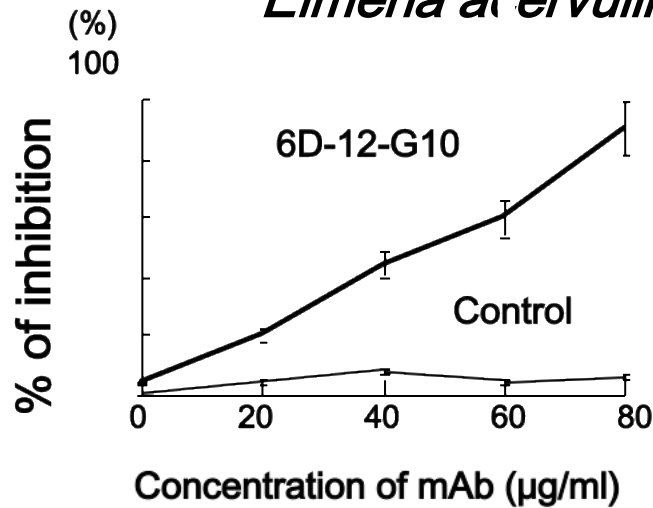
6D-12-G10



Eimeria acervulina



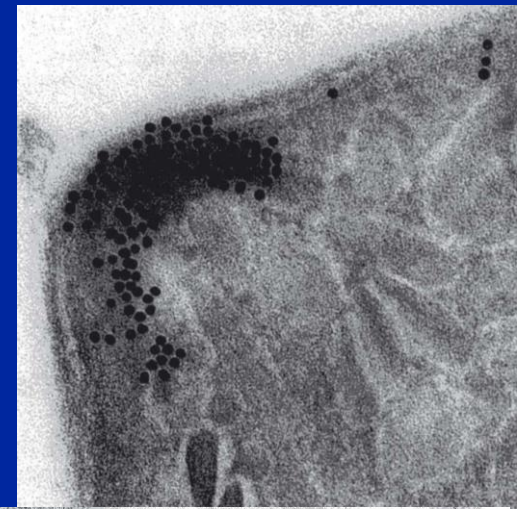
Cryptosporidium parvum



Inhibition test of sporozoite invasion *in vitro*

(Sasai et al., J. Parasitol., 1998; Matsubayashi et al., Vet. Parasitol., 2005, others)

The chicken monoclonal antibodies which recognized the apicomplexan antigens associated with the invasion to host cells



Eimeria
(all chicken's species)

Toxoplasma

Neospora

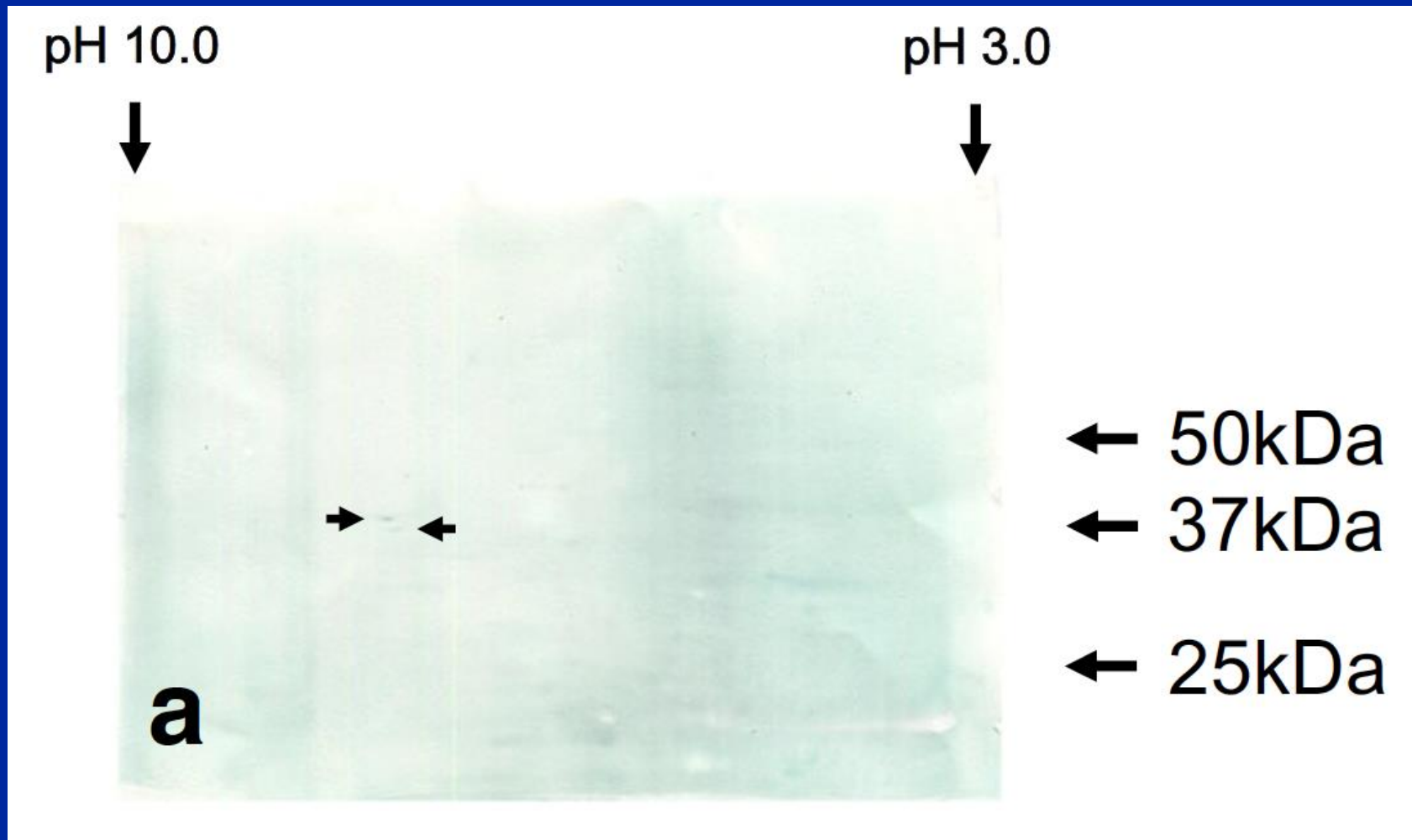
Cryptosporidium

Plasmodium



E. acervulina sporozoite

Two-dimensional Western blot analysis of *E. acervulina* antigens stained with mAb.



LC-MS/MS analysis for the spots

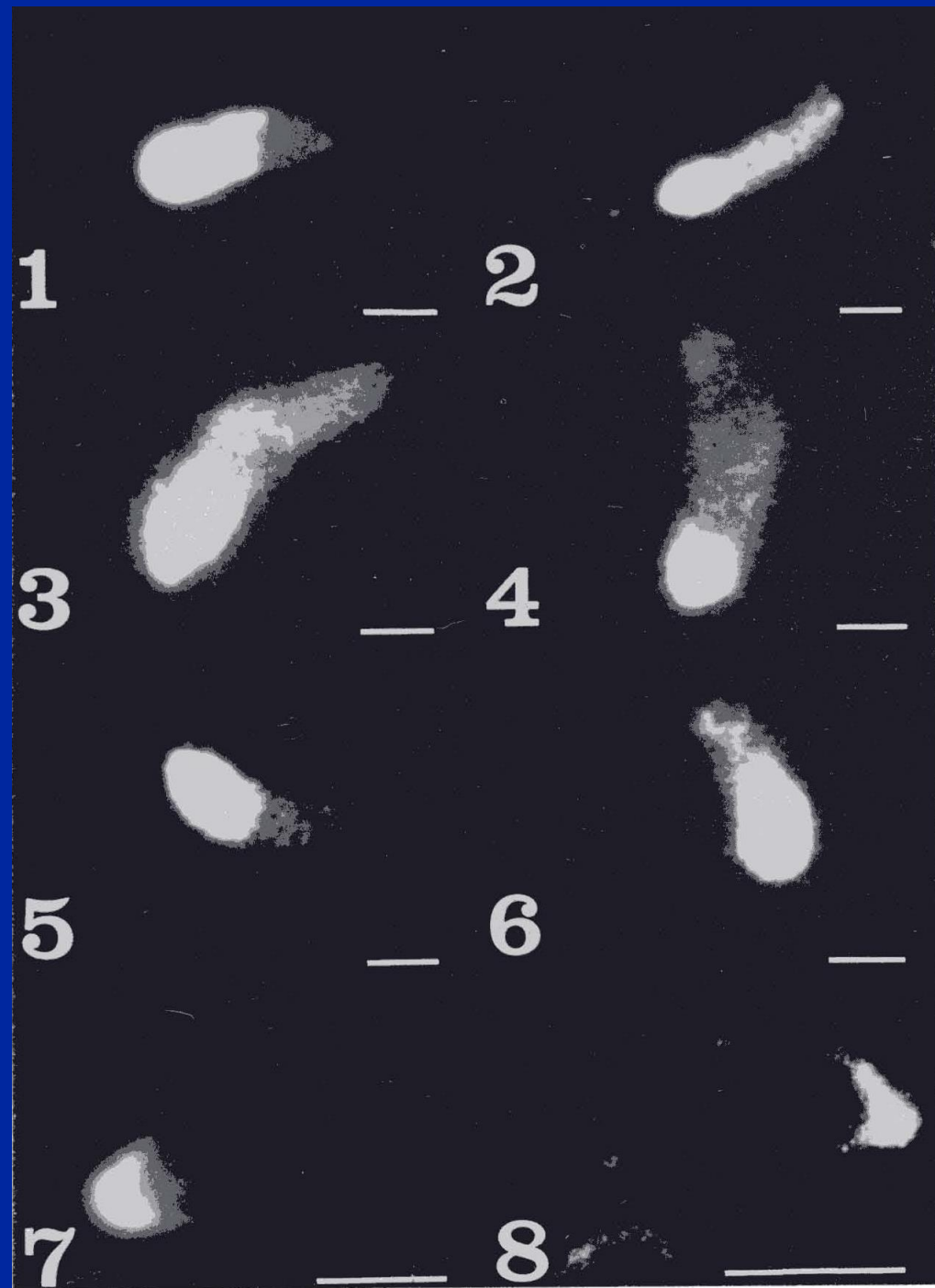
1	MGKEKTHINL	VVIGHVDSGK	STTTGHLIYK	LGGIDKRTIE	KFEKESSEMG
51	KASFKYAWVL	DKLKAERERG	ITIDIALWQF	ETPTFHYTEVI	DAPGHRDFIK
101	<u>NMITGTSQAD</u>	<u>VALLVVPADQ</u>	<u>GGFEGAFSKE</u>	GQTR <u>EHALLA</u>	<u>FTLGVKQMIV</u>
151	GINKMDATTP	EKYSES <u>R FNE</u>	<u>IQAEVSR</u> YLK	TVGYNPE <u>KVP</u>	<u>FVPISGFMGD</u>
201	<u>NMVEK</u> STNMP	WYKGKTLVEA	LDSVEPPK <u>RP</u>	<u>SDKPLRLPLQ</u>	DVYK <u>I</u> GGIGT
251	<u>VPVGRVETGI</u>	<u>LKPGMVVTF</u> A	<u>PTGLQTEVKS</u>	VEMHHTQLEQ	AVPGDNVGFN
301	VKNVSVKDVK	<u>RGHVASDSKN</u>	<u>DPAK</u> GAASFQ	AQVIVLHHPG	QINPGYTPVL
351	DCHTAHISCK	FAELEKRLDR	RSGKALEDNP	KFIKSGDAAI	VKMEPSKPMC
401	VESFIEYPPL	GRFAVRDMKQ	TIAVGV <u>IKAV</u>	EKKEAGGKVT	KSAQKAAGKK

The protein in spots was identified

as *E. acervulina* **EF-1 α** .

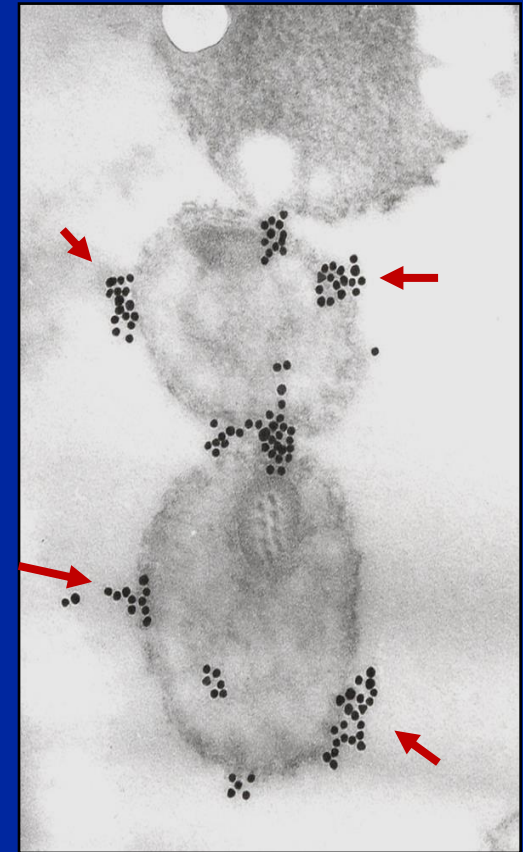
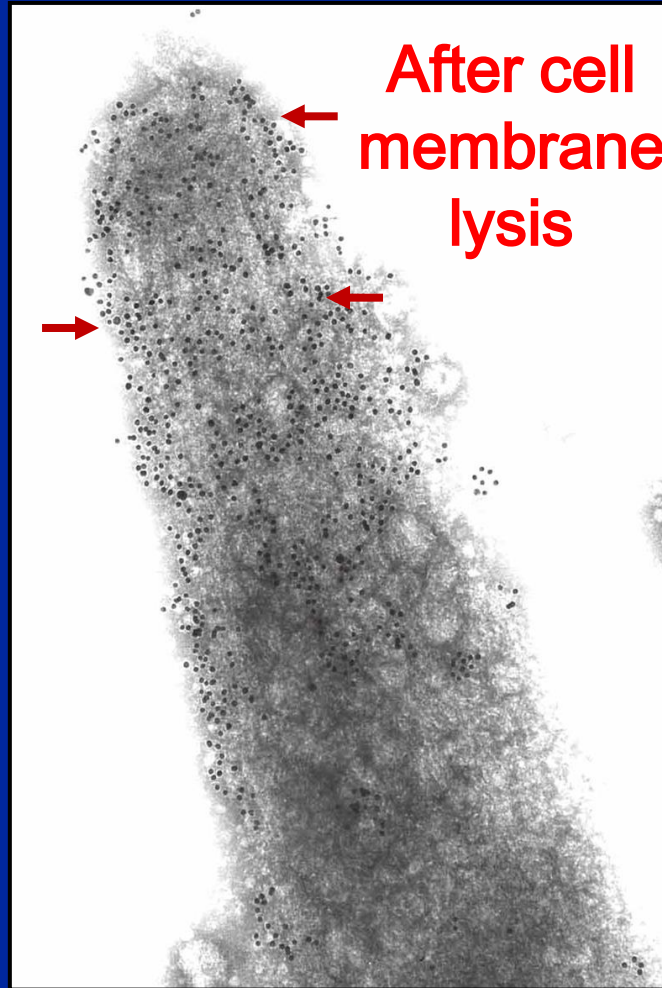
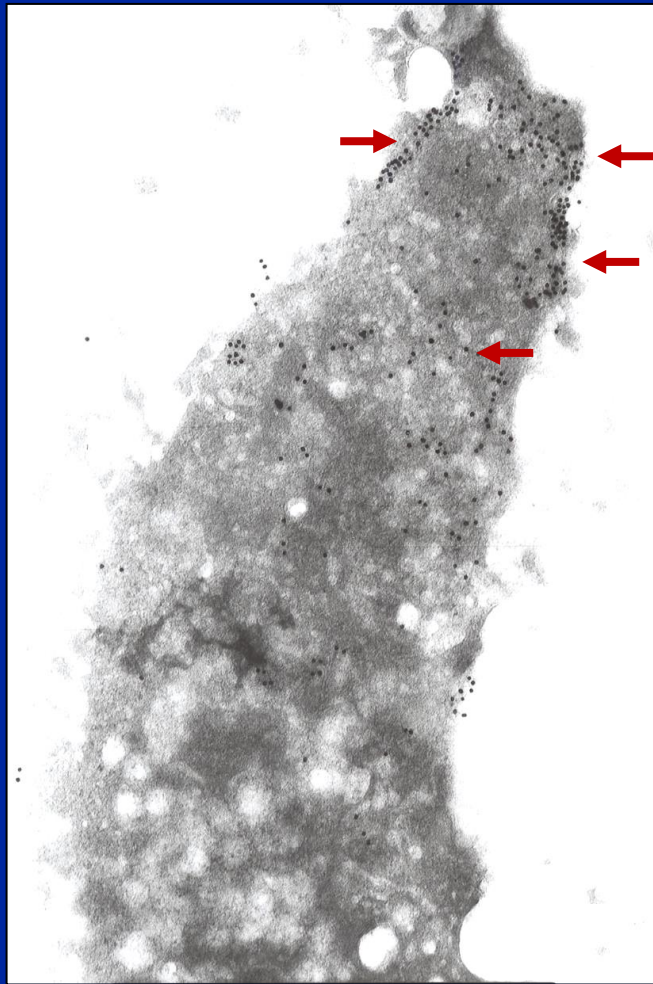
The chicken monoclonal antibodies which recognized the apicomplexan antigens associated with the invasion to host cells

1. *E. brunetti*
2. *E. maxima*
3. *E. mitis*
4. *E. necatrix*
5. *E. praecox*
6. *E. tenella*
7. *Toxoplasma*
8. *Neospora*



Immunoelectron microscopy with mAb 6D-12-G10

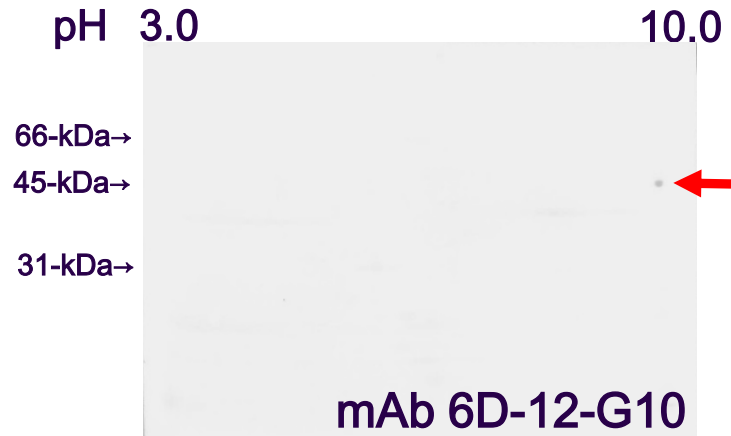
J Biol Chem. 2013



Cross section
of tip

Localization matches the cytoskeleton, Of *Cryptosporidium sporozoite* but is transmembrane

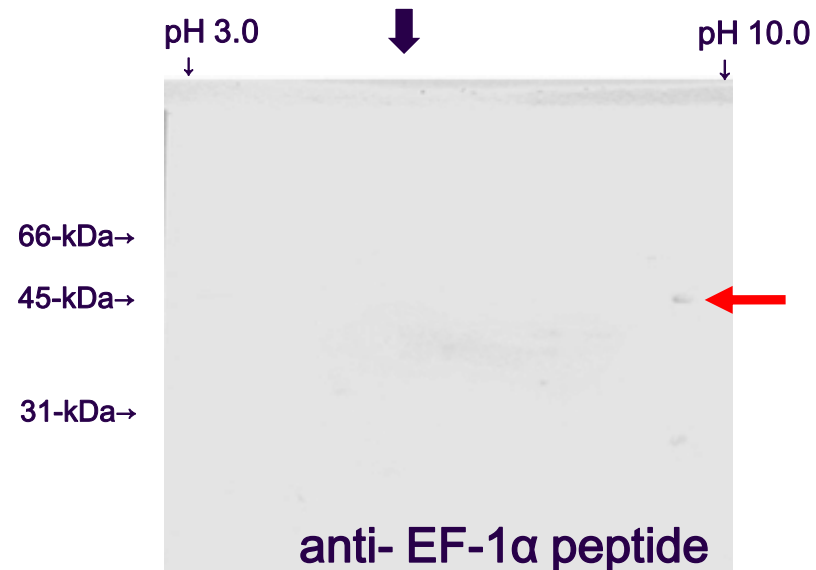
Identification of antigen



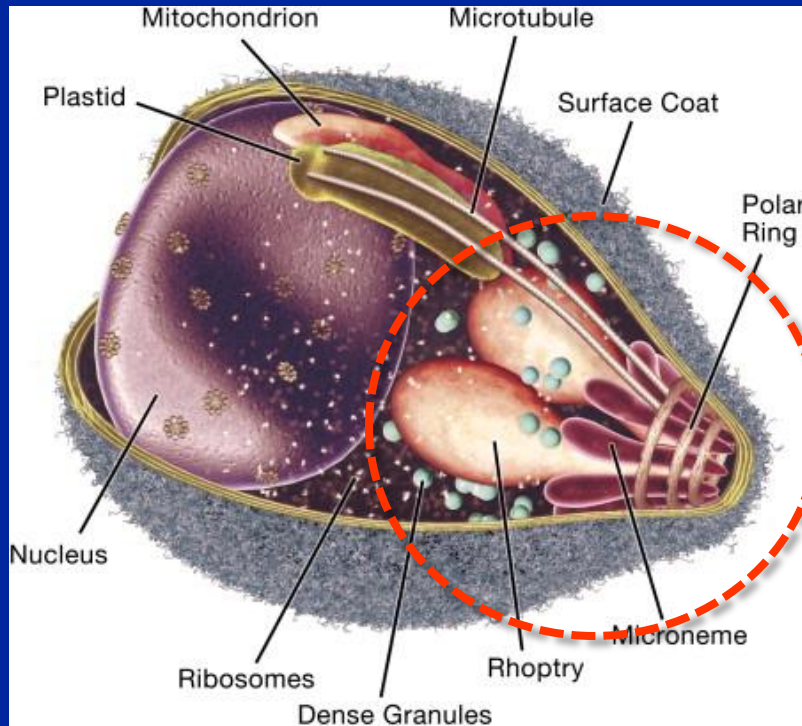
Identification by LC-MS / MS

Protein (organism)	molecular mass [kDa] / pI	sequence coverage
Elongation factor 1α (<i>C. parvum</i>)	48.13 / 8.95	55%

- 1.3kbp protein without intron
- Preparation of peptide antibody with internal sequence



Malaria merozoite



Alan F. Cowman et al. /j.cell.2006

Blood stage Merozoites are weak against immune attack and drugs

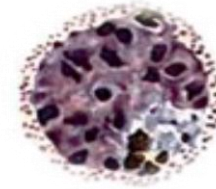


It is a target of vaccine and the drug development

There is no conoid-like structure in *Malaria* same as *Cyptosporidium*

Check the cross-reactivity of anti-conoid Mab

Reactivity with chicken monoclonal antibody



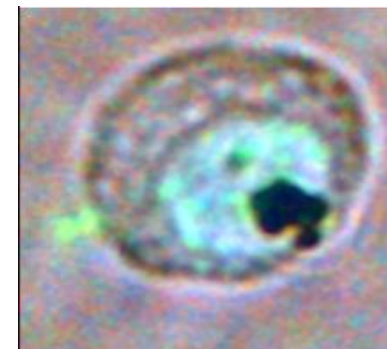
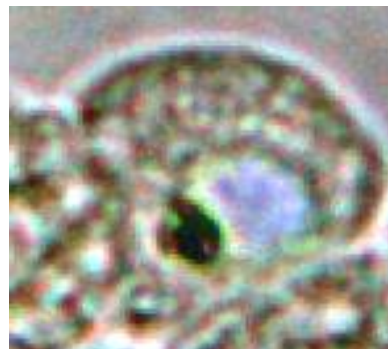
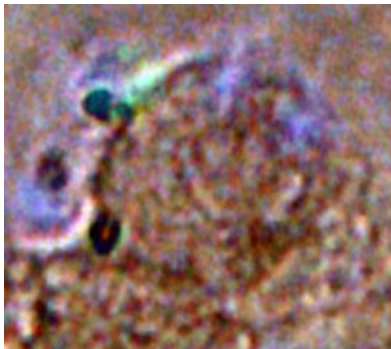
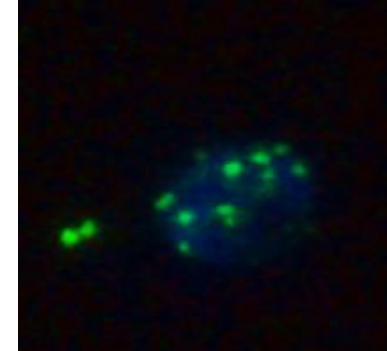
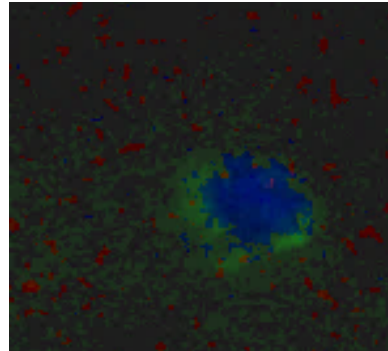
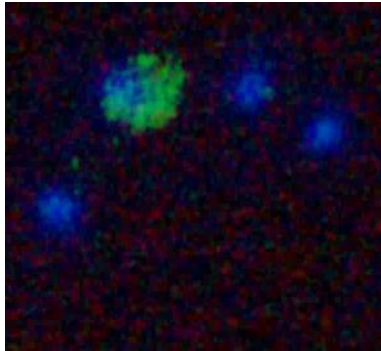
Merozoites

**Ring form
(After
invasion)**

Trophozoites

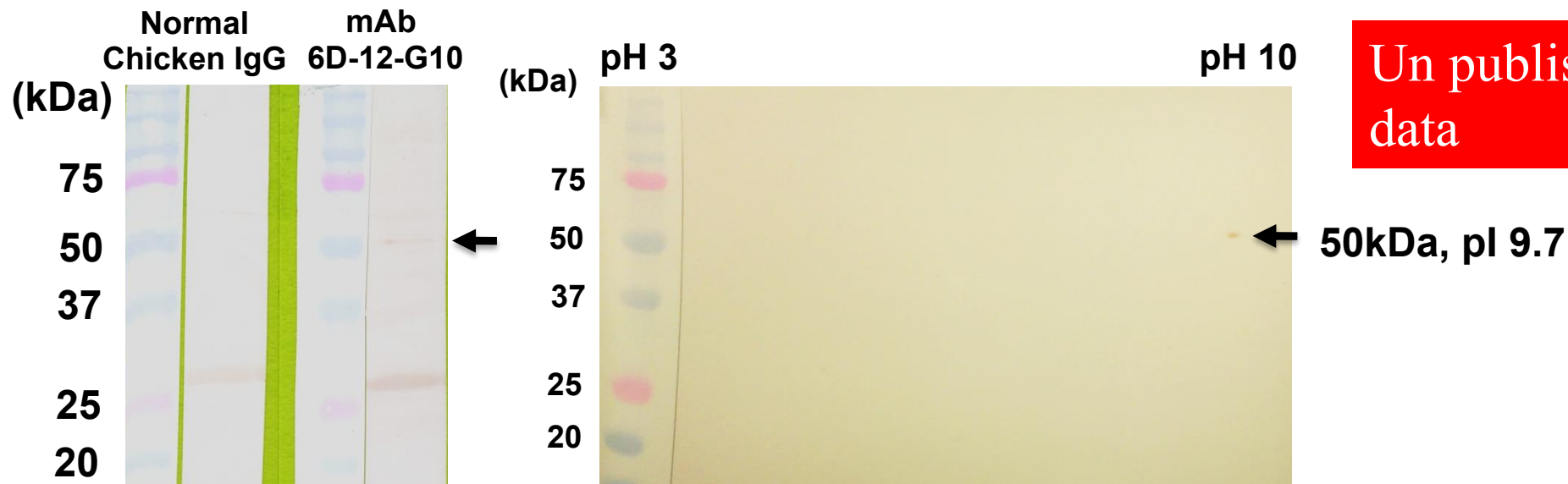
**Schizogony
(Division /
differentiation)**

Merogony

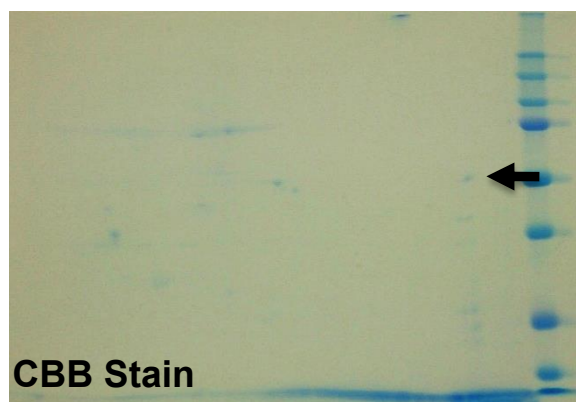


Un-
published
data

Reactivity with chicken monoclonal antibody



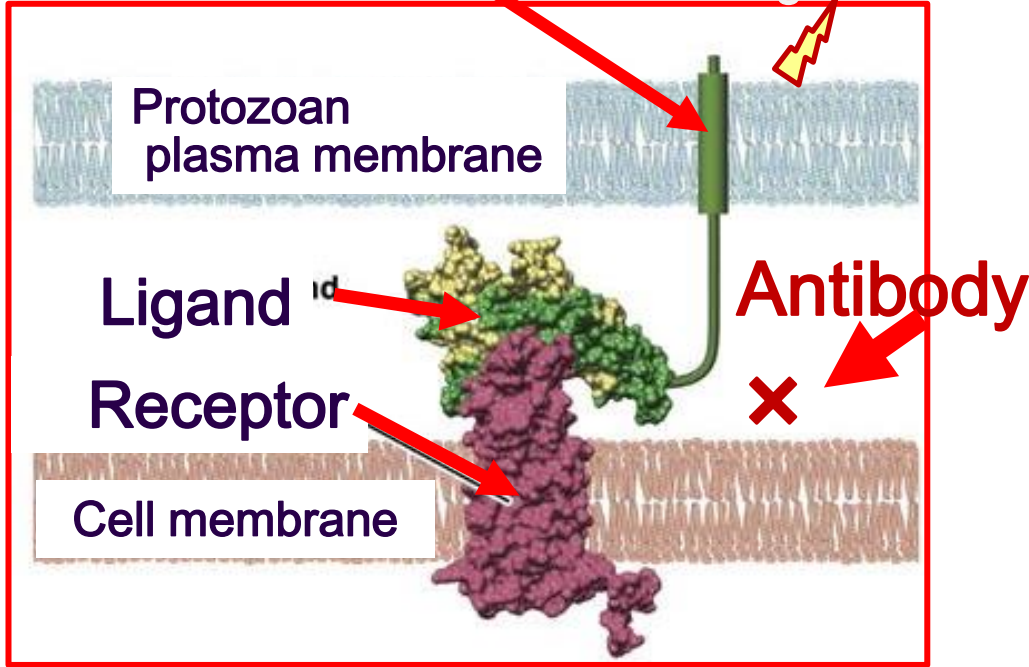
Protein (organism)	molecular mass [kDa] / pI	Database accession no.	sequence coverage	Score
Elongation factor 1 alpha (EF1α) (<i>P. vivax</i>)	48.955 / 9.12	XP_001616354	20 %	273



1	MGKEKTHINL	VVIGHVDSGK	STTTGHI IYK	LGGIDRRRTIE	KFEKESAEMG
51	KGSFKYAWVL	DKLKAERERG	ITIDIALWKF	ETPRYFFTVI	DAPGHKDFIK
101	NMITGTSQAD	VALLVPAEV	GGFEGAFSKE	GQTKEHALLA	FTLVGKQIVV
151	GVNKMDTVKY	SEDRYEEIKK	EVKDYLLKVG	YQADKVDVIP	ISGFEGDNLI
201	EKSDKTPWYK	GRTLIEALDT	MEPPKRPYDK	PLR IPLQGVY	KIGGIGTVPV
251	GRVETGILKA	GMVLNFAPSA	VVSECKSVEM	HKEVLEEARP	GDNIGFNVKN
301	VSVKEIKRGY	VASDTKNEPA	KGCSKFTAQV	IILNHPGEIK	NGYTPVLDCH
351	TSHISCKFLN	IDSKIDKRSG	KVVEENPKAI	KSGDSALVSL	EPKKPMVVET
401	FTEYPPLGRF	AIRDMRQTIA	VGI IKSVEKK	EPGAVTAKAP	AKK Detected peptide

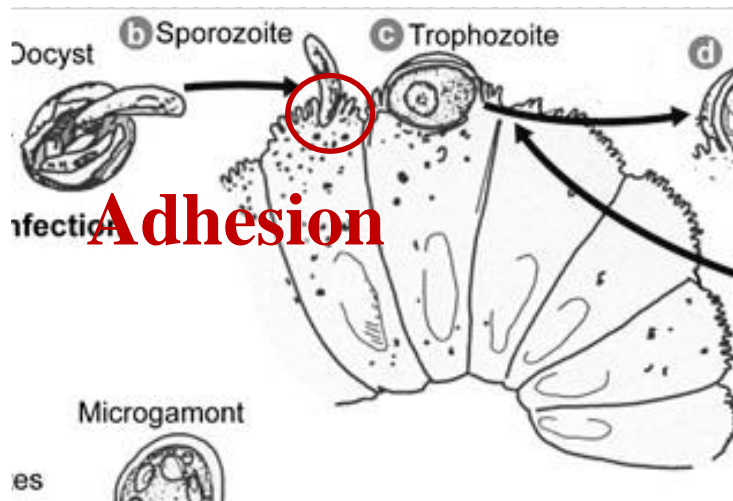
C. parvum Elongation factor 1 α (Ef-1 α)

Discussion



When synthesizing proteins from RNA, **Ef-1 α** is a protein that promotes polypeptide chain elongation.

Motor proteins that react with the cytoskeleton (such as plant cells)



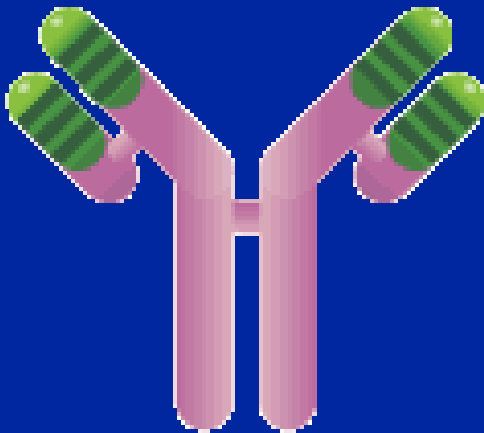
It is thought that it contributes to signaling that causes parasite body movement and leads to active invasion

◆ **Treatment for Apicomplexan parasites using
Chimaera antibody**

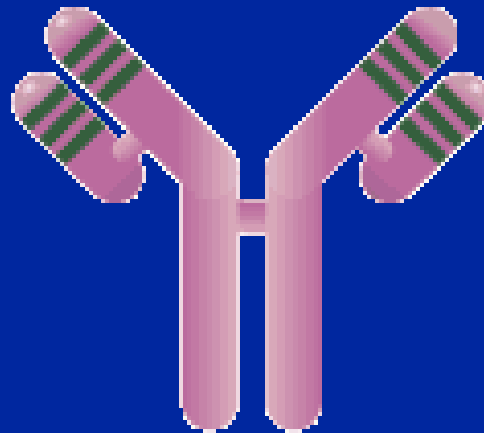
Safety for human is going up



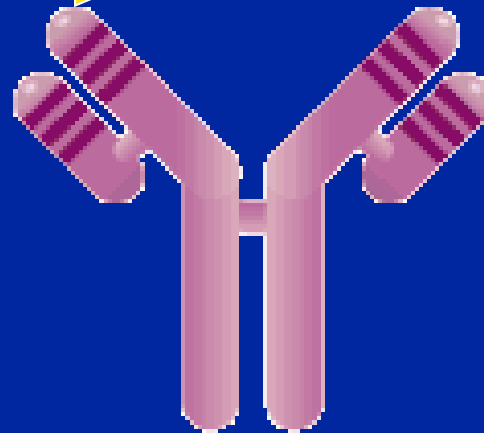
**Chicken
Monoclonal
Antibody**



**Chimaera
recombinant
Antibody(66%)**



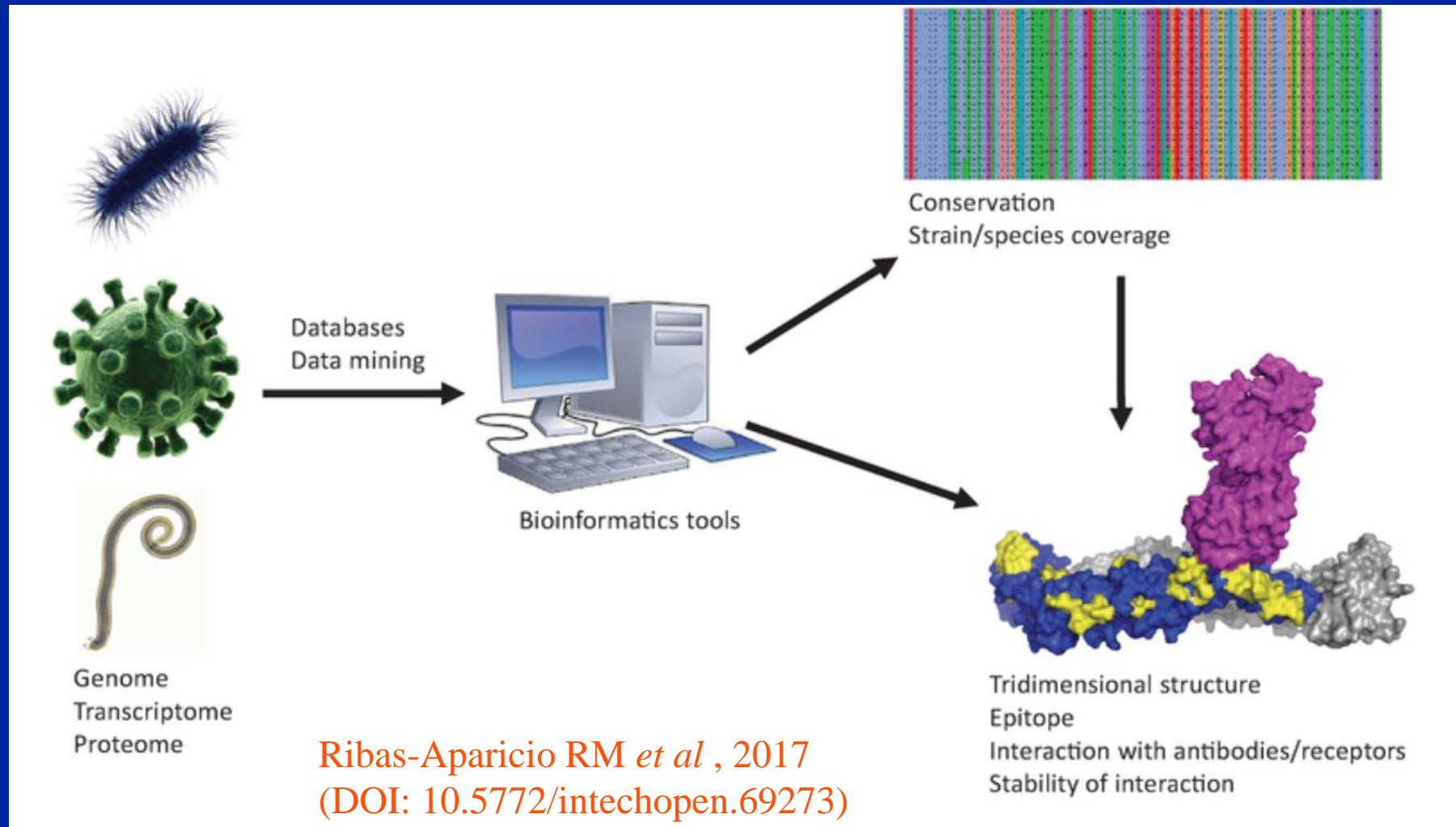
**Chimaera
recombinant
Antibody(90%)**



**Hominized
Recombinat
antibody**

Chicken monoclonal antibody

→ Making of Recombinant vaccine



Our future Targets

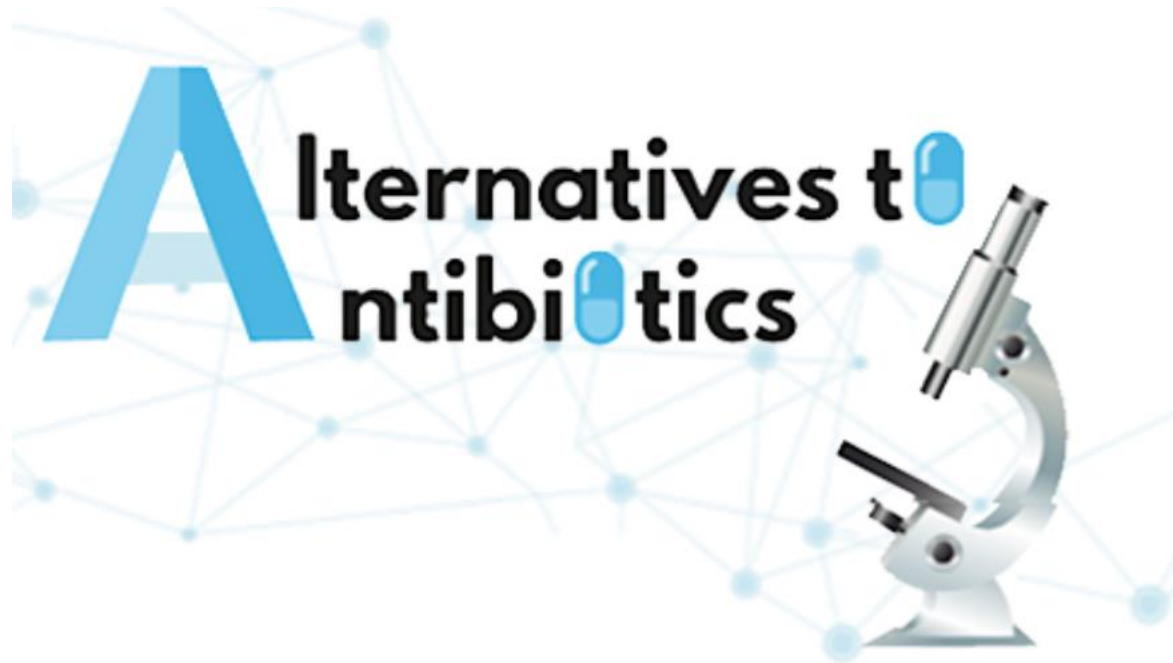
- Transitions in morphological forms and rapid development of the asexual schizonts of *Eimeria tenella* through serial passaging in chicks. (Infect Genet Evol. 2019 Nov)
- Novel Characteristics of Mitochondrial Electron Transport Chain from *Eimeria tenella*. (Genes (Basel). 2019 Jan)
- First surveillance and molecular identification of the *Cryptosporidium skunk* genotype and *Cryptosporidium parvum* in **wild raccoons** (*Procyon lotor*) in Osaka, Japan. (Parasitol Res. 2018 Nov)

Wild raccoons

**Not domestic
Risk of Rabies
& other serious
Zoonosis**



Our Goals



**Alternatives to Antibiotics
Challenges and Solutions in Animal
Health and Production**



Artificial Intelligence in Medicine Market

Global Opportunity Analysis and Industry Forecast, 2018-2025



Acknowledgments

Dr. Kim Jin-Kyoo (Changwon National Univ, Korea)

Dr. Tani H, Dr. Furuya M, Students in my Lab.

Dr. Wergin WP (USDA, Beltsville, USA)

Dr. Fayer R. (USDA, Beltsville, USA)

Dr. Matsuda H (Hiroshima Univ.)

Dr Hemphill (University of Berne, Switzerland)