SELECTION OF A PRECOCIOUS LINE OF *EIMERIA MAGNA* AND EVALUATION OF IT AS A VACCINE COMPONENT (ABSTRACT)

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Abstract
+
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Selection of A Precocious Line of *Eimeria Magna* and Evaluation of It as A Vaccine Component

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ABSTRACT

Coccidiosis is an important parasitic disease in rabbits. Bunnies are usually infected with several coccidia species in the genus of *Eimeria,* resulting in diarrhea, weight loss, and death. Although chemotherapy has been widely used for the controlling of coccidiosis, alternative approaches have to be developed as more and more resistant strains have appeared and concerns of meat safety because of drug residue is growing. In this study, coccidia species infecting rabbits and the infection rate were investigated in Zhang Jiakou city, Hebei province. Strains of *Eimeria magna* were isolated in coccidian-free rabbits using the single oocyst isolation technique, a precocious line was selected, and the feasibility using the precocious line as a vaccine component was evaluated. The research results were as follows: 1) Single oocyst isolation: six strains of *E. magna* were isolated from Zhang Jiakou of Hebei province, Wuji of Hebei province, Leshan of Sichuan province, Zhoushan of Zhejiang province, Kunming of Yunnan province, Nanjing of Jiangsu province. Among 18 coccidia-free rabbits inoculated with a single oocyst of *E. magna,* 16 rabbits shed oocysts, isolation rate of the species was 88.9%; no difference in oocyst production was found among different geographical strains. 2) Pathogenicity of the parent strain: The patent period and peak of oocyst excretion were consistent but oocyst outputs varied with different inoculation doses, rabbits inoculated with 5×10^3 oocysts produced the highest number of offspring oocysts. Compared with the control group, infection groups presented clinic syndrome and lesions to different degree, affected rabbits were anorexia, weight loss, diarrhea, decreased appetite and drinking, as well as excreting soft faeces and loose stool, these changes were rather obvious in the 1×10^4 and 1×10^5 groups, whose tissue sections showed that villi were damaged to different degrees and microvilli destroyed. 3) Selection of a precocious line: 45 days coccidian-free rabbits were inoculated with oocysts of 6 original strains of *E. magna* and the first newly developed oocysts recovered from the intestine and stomach were used for infection of other rabbits. The prepatent period was shortened after 20 passages from 156 to 132 hours, shortened by about 20 h and remained stable after 5 passages without selection pressure. Morphologic features of precocious line oocysts differed from those of original strains but difference is not significance in size; sporulation time of the precocious line was shorter than that of original strains, by 2~4 hours. The oocyst production of original strains is about 536 times higher than that of the precocious line, which was less pathogenic and had reduced reproductive capacity. The pathogenicity and immunogenicity study showed the precocious line was attenuated and remained the immune-protective potent of its parent strain. 4) Phylogenetic analysis: 18S rDNA of the precocious line of *E.magna* was respectively aligned with that of the parent strain of *E. magna* and those *E.magna* in the Genbank, identity was 99.8%; the ITS-1 region1 gene of the precocious line of *E.magna* was also aligned with that of the parent strain of *E.magna* and those in the Genbank, identity is 94.7% and 93.1%, respectively. The phylogenetic tree showed that 18S rDNA and ITS-1 region of the precocious line, parent strain of *E.magna* and *E.magna* strains in the Genbank formed a monophyletic cluster. 5) Endogenous development of the precocious line: The endogenous stages mainly parasitized the jejunum and ileum, including three asexual generations and one sexual generation. Two types of meronts were observed in each asexual generation. The first asexual generation of meronts occurred 48h post inoculation (p.i.), the second was 72 h p.i. and the third was 96 h p.i. The macrogamonts and microgamonts took place in 96~108h p.i. The mature oocysts were found 120 h p.i. This is the first report of precocious line selection of rabbit coccidia in China and the selected precocious line was attenuated and its immunogenicity was maintained, indicating its feasibility as a vaccine component.

Key Words: Rabbit, Eimeria Magna, Anti-coccidial Vaccine, Precocious Selection

Selection of a precocious line of *Eimeria magna* and evaluation of it as a vaccine component

Reporter: Xun Suo

National Animal Protozoa Laboratory &
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China Agricultural University

Aug 28, 2013 Bali, Indonesia

Coccidia of rabbits

Species	Morphology	localisation	Pathogenicity	Immunogenicity
E. intestinals		Lower jejunum and ileum	Highly pathogenic	high
E. flavescens		Small intestine and caecum	Highly pathogenic	low
E. stiedai		Liver	Mildly pathogenic	
E. media		Duodenum and jejunum	Mildly pathogenic	middle
E. magna		Jejunum and ileum	Mildly pathogenic	middle
E. piriformis		Colon	Mildly pathogenic	low
E. irresidua		Jejunum and ileum	Mildly pathogenic	middle
E. perforans		Duodenum,jejunum and ileum	Slightly pathogenic	
E. exigua		Small intestine	Slightly pathogenic	
E. vejdovsky		Ileum	Slightly pathogenic	
E. coecicola		Appendix, sacculus rotundus, PPs patches	No pathogenic	

Coccidia of rabbits isolated by my group

E. stiedai

E. magna

E. media

E. piriformis

E. coecicola

E. intestinalis

E. irresidua

E. exigue

E. flavesens

E. perforans

Economic importance of coccidiosis in rabbits

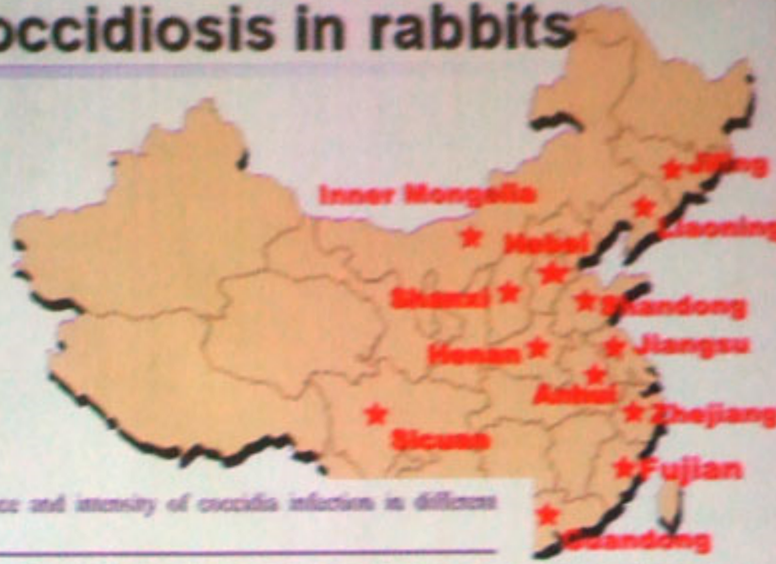

Table 1 Prevalence and intensity of coccidia infection in different regions of China

Locality	Exami no.
North China	200
South China	120
Southwest China	120
Northwest China	20
Northeast China	20

Table 2 Percentage of faecal samples infected with each coccidia species in rabbits in China

Species	Examined no.	Positive no.	Prevalence (%)	OPG
E. magna	496	138	29.8	43,400
E. media	496	156	31.3	17,200
E. coecicola	496	28	5.83	132,400
E. intestinalis	496	71	14.8	43,400
E. perforans	496	169	35.2	46,200
E. irresidua	496	93	19.4	9,200
E. piriformis	496	13	2.71	3,200
E. flavescens	496	22	4.58	9,600
E. stiedai	496	2	0.42	1,400
E. exigua	496	1	0.002	2,800

Yin, et al. *Parasitol Res*, 2011

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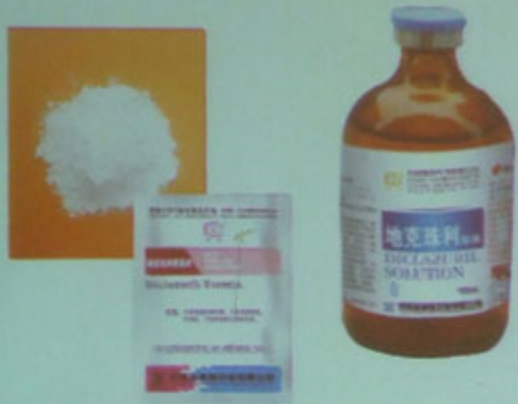
Strategies for the control of coccidiosis in rabbits



- Drug resistance
- High economic cost
- Public health security

Rabbits industry needs an alternative mean to control coccidiosis

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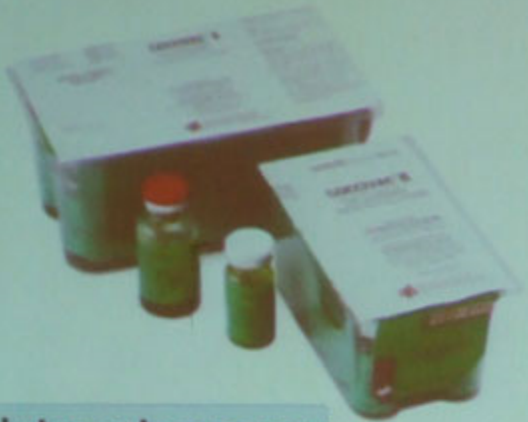
Strategies for the control of coccidiosis in rabbits

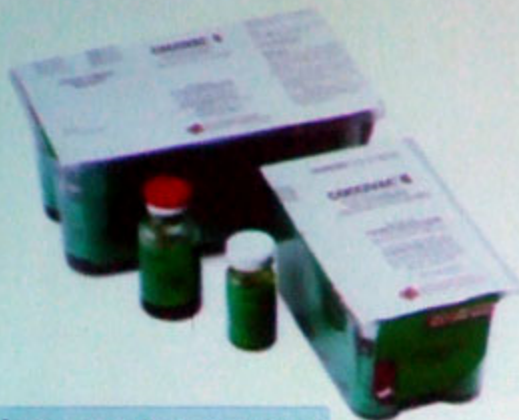
In other species : *Control of avian coccidiosis with vaccines and chemical medicines*



Intervet.com.mx

In 1952, **CocciVac** was introduced into the market, then came
Immucox in 1982,
Paracox in 1989,
Livacox in 1992,
Supercox in 2006…

Strategies for the control of coccidiosis in rabbits



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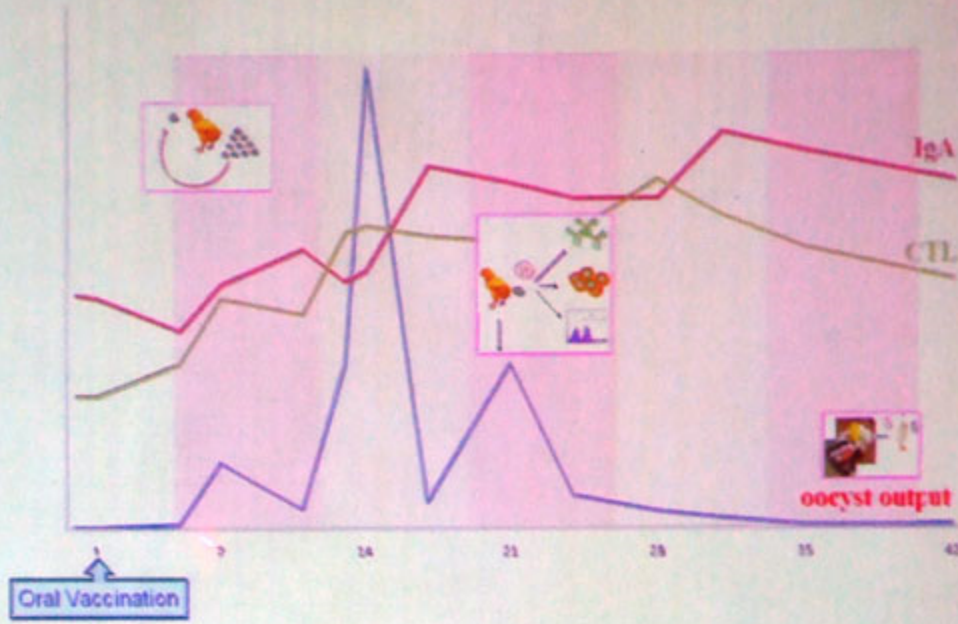
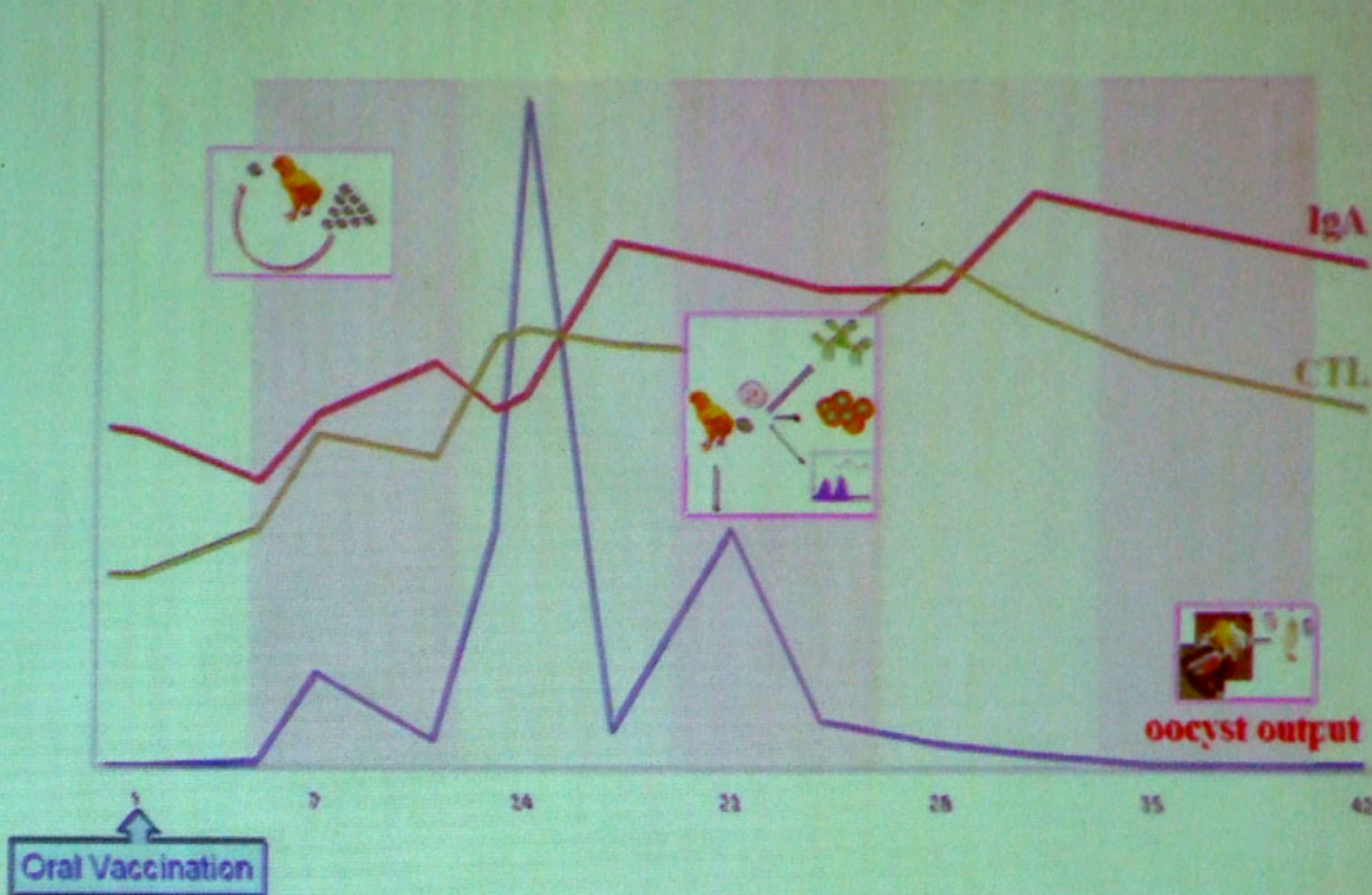
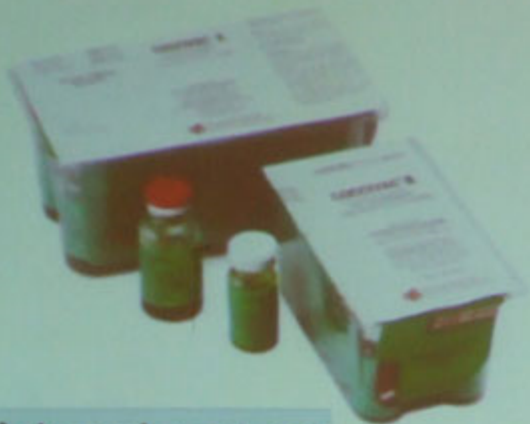


Example of control of avian coccidiosis in the chickens with a multivalent vaccine



Example of control of **avian** coccidiosis
in the chickens with a multivalent vaccine

Strategies for the control of coccidiosis in rabbits



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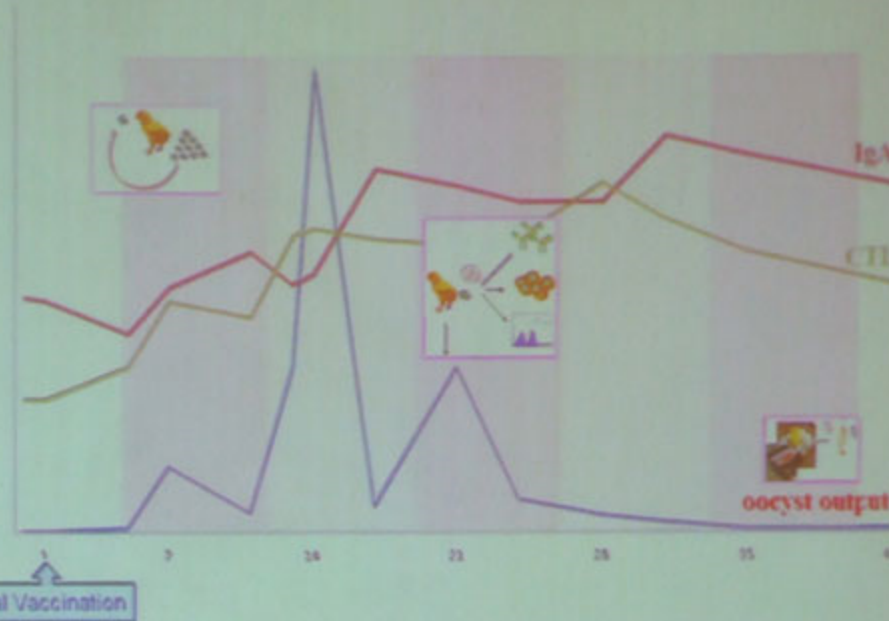

Currently, no available vaccines are used in rabbit coccidiosis control

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PRECOCIOUS LINE SELECTION

Field samples

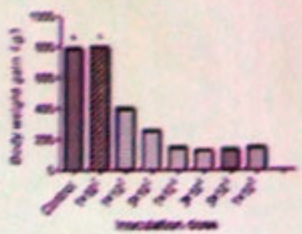
↓ Single oocyst isolation

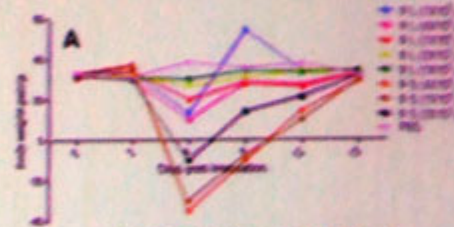
Original strain → **Pathogenicity test**

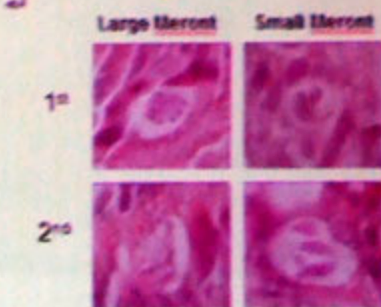
↓ Pressure passage

Precocious line

- → **Pathogenicity test**
- → **Immunogenicity test**
- → **Phylogenetic analysis**
- → **Endogenous development**







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Single oocyst isolation of *E. magna* from filed samples

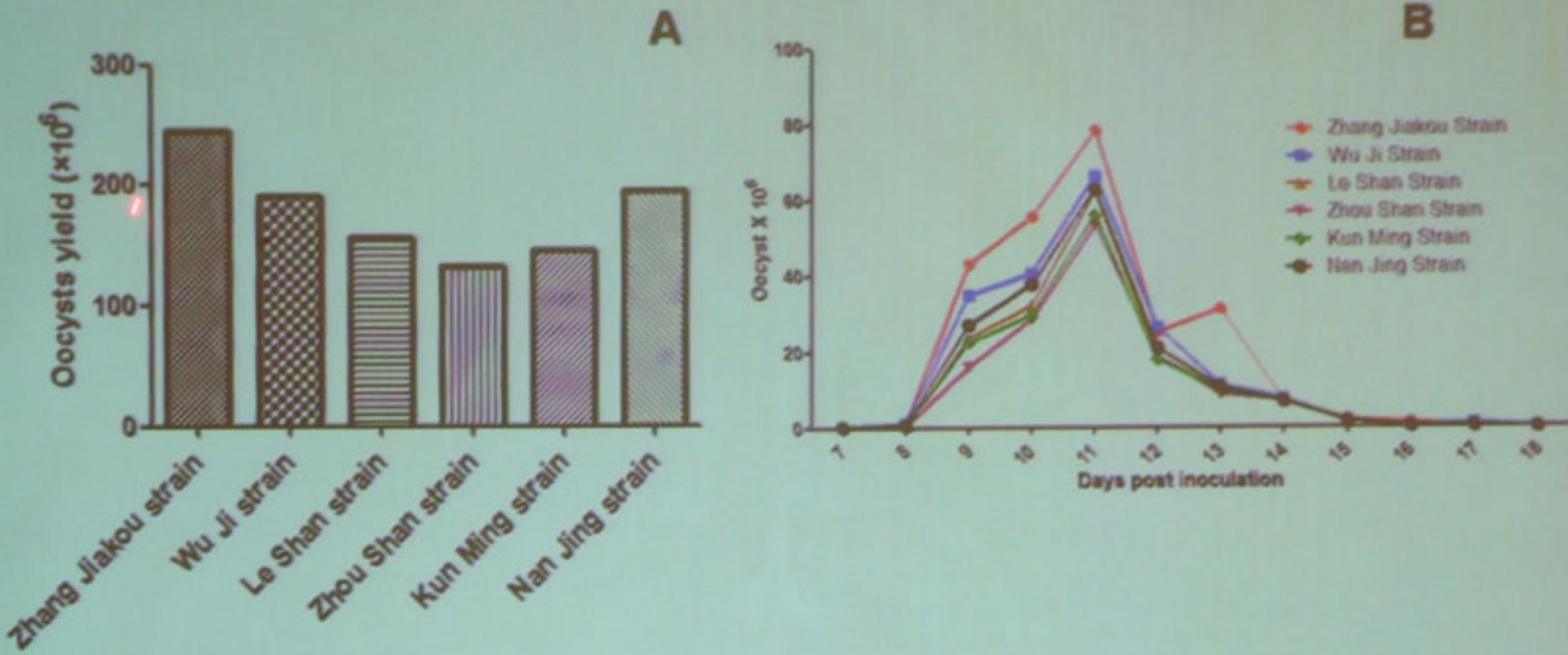

Fig.1 Total (A) and daily (B) oocyst yield during patent period after inoculation with different strains.

Pathogenicity of the parent strain

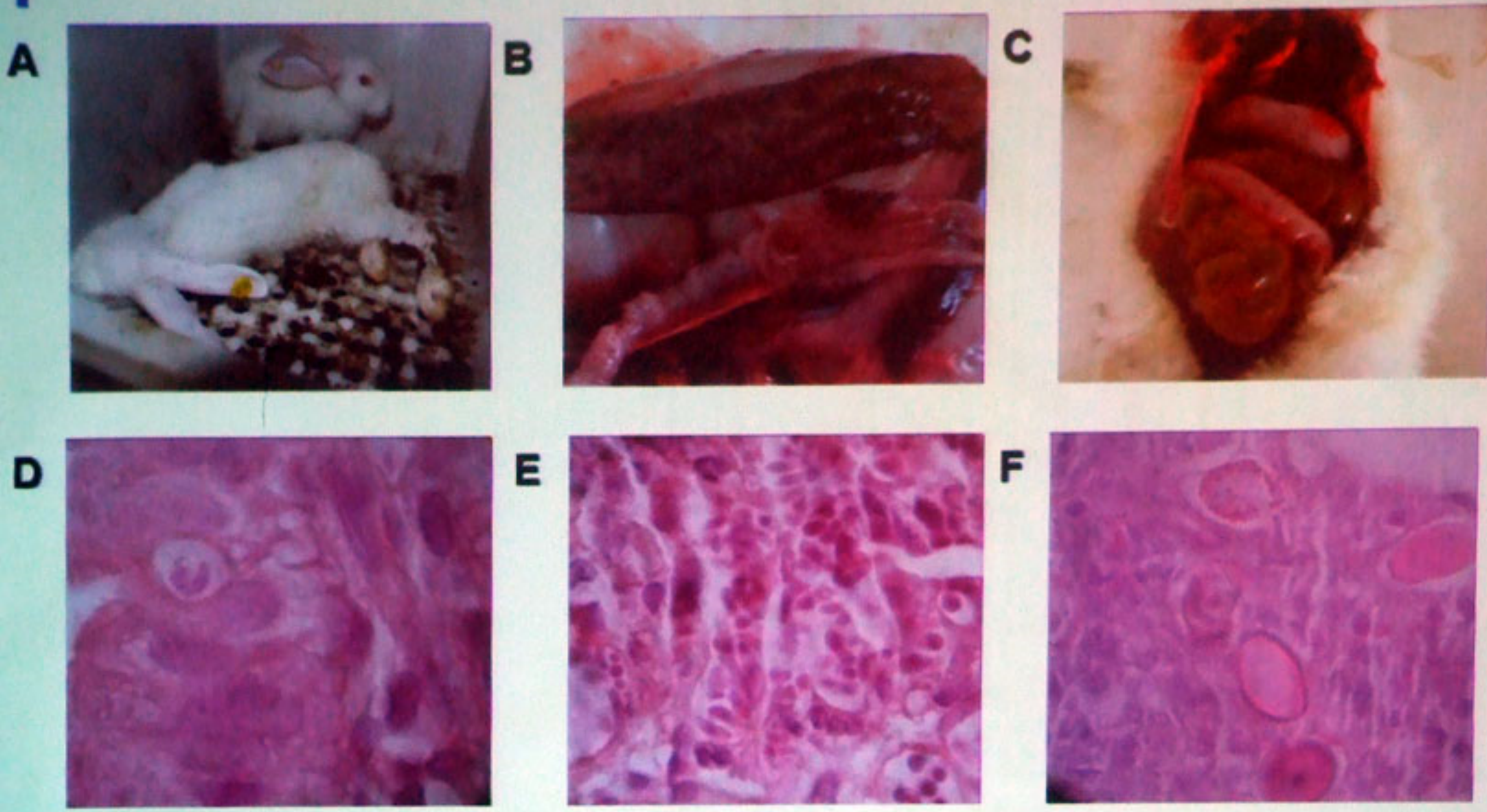

Fig.2 Clinical symptoms (A), postmortem examination(B,C) and histopathological changes (D,E,F) of rabbit infected with *E. magna* parent strain.

Pathogenicity of the parent strain

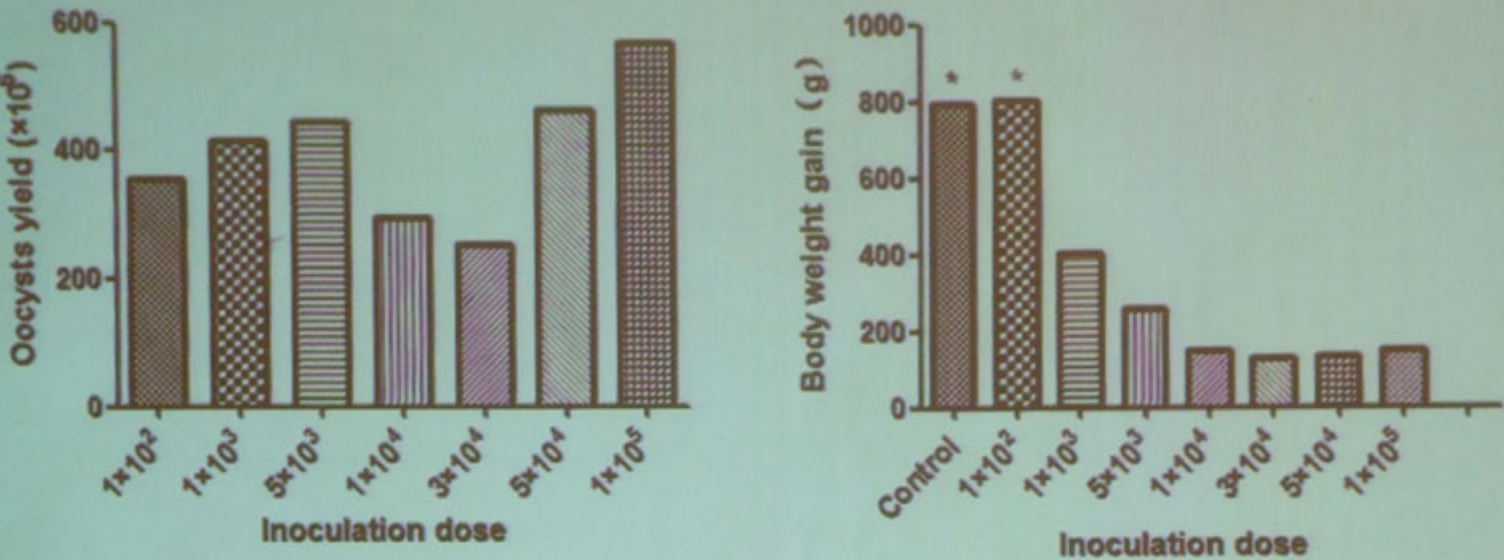

Fig.3 Total oocyst yield (A) and body weight gain (B) after inoculation with different doses of parent strain 24 D.P.I.

Selection of a precocious line of *E. magna*

Table 1. Selection of Precocious line of *E. magna*

Passage time	Passage No.	Animal No.	Inoculation dose/ animal	Oocysts collection	Oocyst excretion time
2011.8.7—8.14	1	4	5000	caecum content	144—156h
2011.9.24—9.30	2	4	5000	caecum content	140—153h
2011.10.6—10.13	3	4	5000	Stomach and caecum content	149—158h
2011.10.18—10.25	4	4	5000	Stomach content	147—149h
2011.11.2—11.8	5	4	8000	Stomach content	148—156h
2011.11.11—11.17	6	4	6000	caecum content	132—141h
2011.11.21—11.28	7	4	1mL	caecum content	165h
2011.11.30—12.6	8	4	5000	Stomach and caecum content	144—152h
2011.12.12—12.19	9	4	5000	Stomach content	144—150h
2011.12.23—12.30	10	4	5000	Stomach and caecum content	149—152h

Selection of a precocious line of *E. magna*

Table 1. selection of Precocious line of *E. magna*

Passage time	Passage No.	Animal No.	Inoculation dose/ animal	Oocysts collection	Oocyst excretion time
2012.1.3—1.10	11	4	5000	Stomach content	153—161h
2012.1.13—2.20	12	4	5000	caecum content	148h
2012.2.23—2.29	13	4	1mL	caecum content	153h
2012.3.5—3.12	14	4	10000	caecum content	151h
2012.3.15—3.21	15	4	10000	caecum content	147h
2012.3.26—4.2	16	4	10000	Stomach content	156h
2012.4.2—4.8	17	4	1mL	caecum content	141h
2012.4.12—4.18	18	4	6000	Stomach content	144h
2012.4.22—4.28	19	4	1mL	Stomach content	138h
2012.5.2—5.8	20	4	1mL	faeces	132h

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Selection of a precocious line of *E. magna*

P.S. P.L.

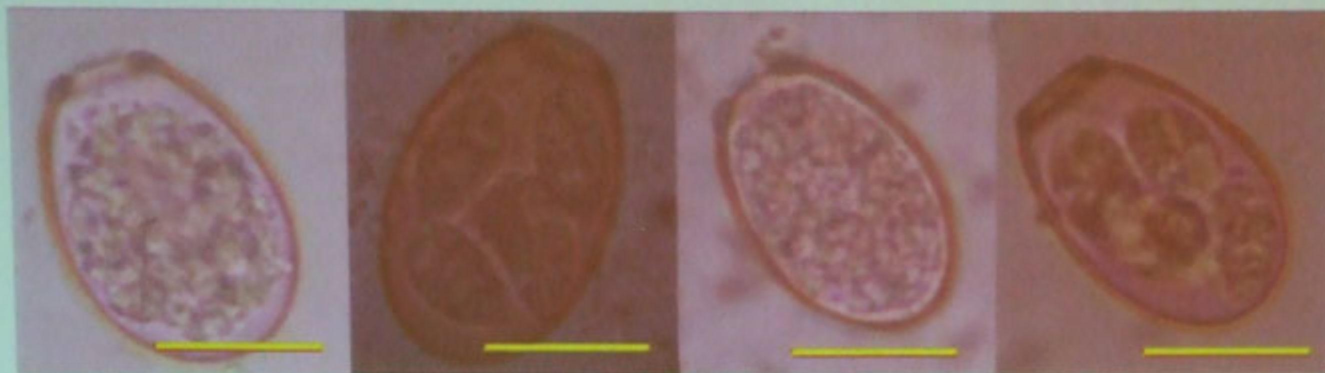


Table 2. Comparison of oocysts shape between parent strain and precocious line

Group	Oocysts size			Sporocysts size		
	length	width	Length/width	length	width	Length/width
Parent strain	35.7±1.67	24.5±1.47	1.46±0.10	13.9±1.25	9.14±1.04	1.55±0.19
Precocious line	33.1±3.03	24.0±2.53	1.39±0.14	14.3±1.54	9.80±1.29	1.48±0.20

Pathogenicity & immunogenicity of the precocious line

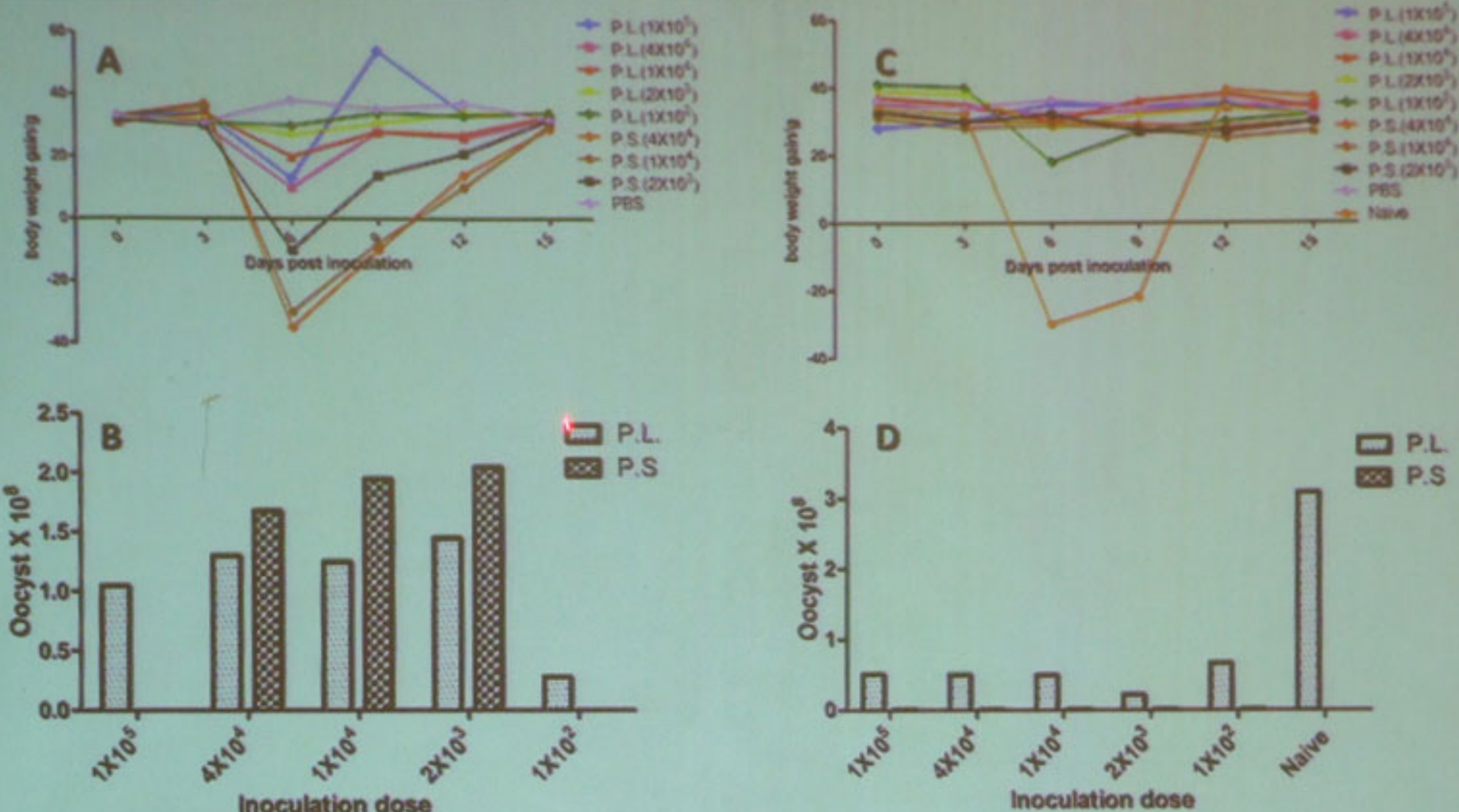
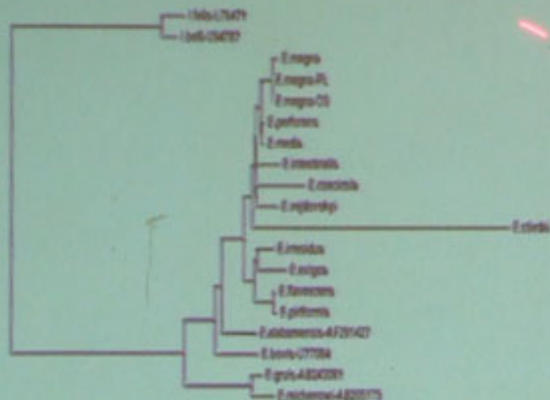
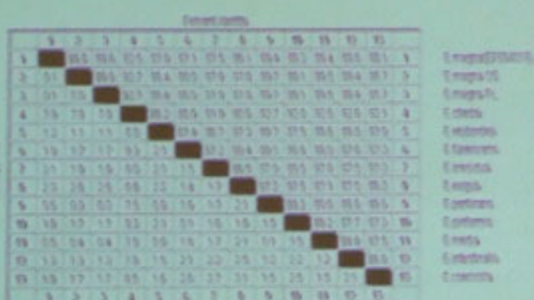


Fig.4 Pathogenicity and immunogenicity of E. magna precocious line. A & B. Body weight gain and total oocysts yield after immunization with different doses of P.L.(precocious line) and P.S.(parent strain). C & D. Body weight gain and total oocyst shedding after challenge with 2×10⁴ oocysts of E. magna P.S. 15 d.p.i.

Phylogenetic analysis of the precocious line

18S rDNA





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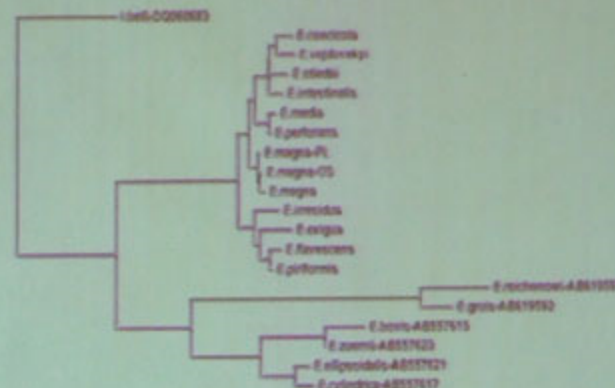

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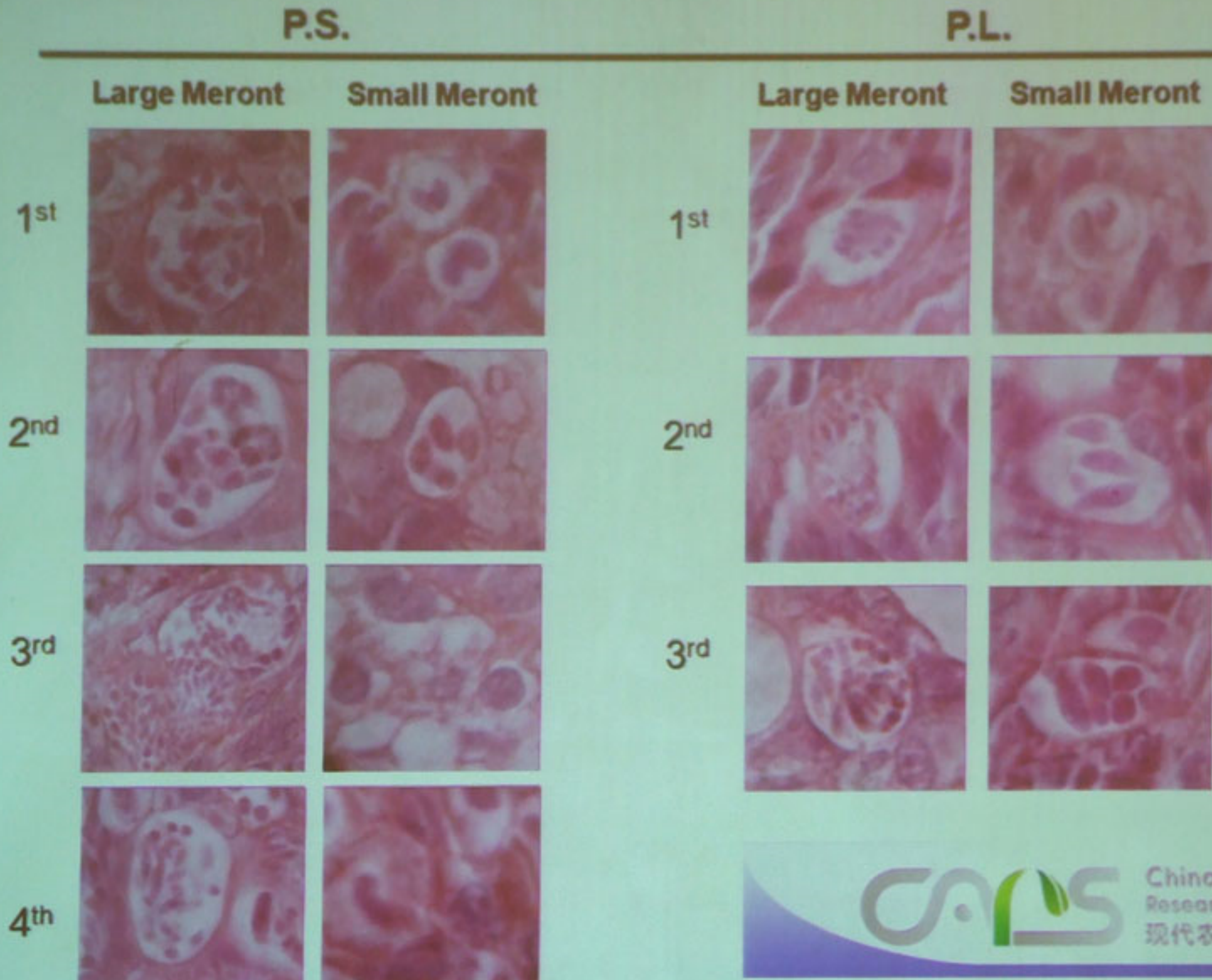
Endogenous development



Endogenous development

Table 3. Comparison of schizogony generation with the parent strain

Asexual generations	strains	localization	h.p.i	Large meront		Small meront		Ratio: Large/small
				Size	No. of schizont	Size	No. of schizont	
1	P.S	villous epithelium	48	7.5~12×5~10	9~18	6~8× 5~7	2~4	1:2
	P.L		36-48	13~20×9~12	8~23	7~10× 7~9	2~4	1:2
2	P.S	villous epithelium	72	8~15×7~11	15~35	6~8×5~7	2~4	1:1
	P.L		60-72	14~23×9~18	18~25	9~13×8~11	2~4	1:1
3	P.S	villous epithelium	96	14~22×9~17	25~75	8~13×6~9	2~6	3:1
	P.L		84-96	12~26×11~22	21~82	9~16×6~12	2~7	3:1
4	P.S	crypts	–	–	–	–	–	–
	P.L		108-120	22~36×13~27	34~105	9~15×7~11	2~6	2:1

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Transgenic rabbit coccidia as vaccine vectors

E. stiedai oocysts expressing YFP and alpha-toxin of *Clostridium perfringens* after 12 sorts and passages in rabbits. Bar=20 μm. (7% fluorescent oocysts in the population)

E. intestinalis oocysts expressing YFP and alpha-toxin of *Clostridium perfringens* after 13 sorts and passages in rabbits. Bar=10 μm. (2.5 % fluorescent oocysts in the population)

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