

CHAPTER V

RESEARCH RESULTS

The follow-up of the course of migrating larvae of *Ascaris suum* in experimentally infected mice (Experiment I), resulted in absolute numbers of larvae recovered by the Baerman method in various organs as presented in Tables 1,1 - 1,5. A Schematic representation is given in Figure 2, regarding the recover from liver and lungs. A peak recovery in the liver is found 5 days after oral infection and in the lungs 7 days after the infection. Since large intermediate periods were used between the actual recovery of the larvae, a second follow-up study was done in nine consecutive days. These results are presented in Tables 2,1 - 2,9 and in Figure 3. Both in the summarizing Table 2,9 and in Figure 3, it can be seen that the peak recovery of larvae from the liver is at day 3 post infection and from the lungs at day 7 post infection. Virtually no larvae were found in other organ systems or in the peripheral blood throughout the observation periods.

The results of experiment II are summarized in tables 3 and 4, representing the larval counts in the livers and lungs at days 3 and 7 post challenge respectively. All animals immunized showed a significantly reduced number of migrating larvae in the livers as compared to the control animals (Wilcoxon's test), whereas the decrease in number of migrating larvae in the lungs was also obvious however no statistically reliable figures can be presented due to the low recovery of larvae from the lungs of control animals (Table 4). The serological response during the immunization and after challenge is presented in Figure 4 with the various antigens applied for immunization as coating antigen in the ELISA double sandwich assay. It

can be seen that cross reactions between the various groups exist (data presented as mean extinction values of five determination) although the homologous system gave the highest antibody titers. Animals immunized with somatic antigens of L2 nearly showed antibody formation. The control animals (group V) did not show antibodies when they were killed 3 and 7 days post infection challenge.

In experiment III protection induced by ES antigen of L2 and L3/4 alone or in combination was studied. The results are given in Tables 5 and 6 for the recovery of migrating larvae in livers (3 days post infection challenge) and lungs (7 days post infection challenge) respectively. A clear protection in all immunized groups was observed as compare to the control groups, including the mice stimulated with Freund's complete and incomplete adjuvant. However the statistical analysis with Wilcoxon's test showed no significance in the values obtained with the numbers of larvae recovered from the livers due to a single animal in each control group with no migrating larvae at all. When these animals were not used in the analysis a highly significant protection ($p < 0.05$) became obvious. The protection varied from 91-95% in the livers to 88-93 % in the lungs. Many animals per group did not show migrating larvae at all (total protection). The serological results with the sera obtained from the mice immunized in this experiment were summarized in Table 7. Good antibody formation was observed in the homologous system and negligible or no antibodies to the heterologous ES antigen. However some cross reactivity was observed when somatic antigens were applied in the ELISA sandwich method.

Results of the cross-immunization study between *Ascaris suum* and *Ascaris lumbricoides* with the help of ES/L2 antigens are presented in Tables 8 and 9 regarding the recovery of migrating larvae. It is obvious that a clear protection occurred in all immunized groups of mice both when larvae were counted in the livers (day 3 post challenge infection) and the lungs (day 7 post

challenge infection). Again single dissonant mice in groups V and VII showing no migrating larvae at all caused difficulties in the statistical analysis in Table 9. If these animals could left out from the analysis significant protection ($p < 0.05$) in all groups would have been shown. The reduction of migrating *A.suum* in the livers was 86 % in the homologous system (group I versus IV and VI). The reduction of *A.lumbricoides* larvae in the livers was 92 % in the homologous system (group III versus V and VII) and 90% in the heterologous system (group II versus V and VII).

Similarly the reduction of migrating larvae of *A.suum* in the lungs was 84% in the homologous system, whereas the reduction of *A.lumbricoides* larvae was 94 % in the homologous system, and 91 % in the heterologous system. Particularly in the lungs quite often no larvae at all were found (total protection) although this phenomenon was also observed in some control animals. The results of the serological examination with the sera of the mice at the end of Experiment IV are summarized in Table 10. ES antigens of L2 *A.suum* are recognized by all immunized groups. The ES antigens of L2 *A.lumbricoides* are particularly recognized by the groups immunized with *A.lumbricoides* antigen. The ES antigen of L3/4 larvae of *A.suum* are poorly recognized by all groups, whereas the somatic antigens of either stage of larvae cross react serologically with all animals immunized.

The results of the immunoblotting (after SDS-PAGE) are visually presented in the Figures 5, 6 and 7. The sera of the mice immunized with ES/L2 antigens of *A.suum* produce a specific couple of two distinct lines representing antigens with molecular weights of 47,4 and 47,2 K Dalton respectively. These lines were not detected with sera from mice immunized with ES/L3/4 antigens. However, the latter reacted specifically with a single antigen with a molecular weight of 46,7 KD (Figure 5). Also immune sera against ES/L2 *A.lumbricoides* gave a one line reaction in immunoblotting when

ES/L2 and ES/L3/4 *A.suum* antigens were separated by SDS-PAGE (Figure 6). In the homologous system however the ES/L2 *A. lumbricoides* did not show any reaction in the concentrations used in that experiment (Figure 6, lines 2-7). In a following experiment the more concentrations serum of mice immunized with ES/L2 *A.lumbricoides* antigen specifically reacted with an ES/L2 *A.lumbricoides* antigen with a molecular weight of 46,1 KD, which was not recognized by the sera of mice immunized with ES/L2 *A.suum* or ES/L3/4 *A.suum* antigens (Figure 7).

Table 12 summarizes the number of mice in the experiment and control groups which were fully protected from infection (no migrating larvae detected). Up to 44.5% (49/110) of mice in the experiment group were protected compared to only 7% of mice in the control group.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 1.1**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 3 p.i. (number of larvae collected)						
	1*	2	3	4	5	total
Liver	168	70	36	30	18	322
Lungs	0	1	0	0	0	1
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	168	71	36	30	18	323

* Individual animals

Table 1.2**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 5 p.i. (number of larvae collected)

	6*	7	8	9	10	total
Liver	225	62	250	210	140	887
Lungs	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	225	62	250	210	140	887

* Individual animals

Table 1.3**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 7 p.i. (number of larvae collected)

	11*	12	13	14	15	total
Liver	160	53	40	20	20	293
Lungs	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	160	53	40	20	20	293

* Individual animals

Table 1.4**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 14 p.i. (number of larvae collected)

	16*	17	18	19	20	total
Liver	0	0	0	0	0	0
Lungs	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	0	0	0	0	0	0

* Individual animals

Table 1.5

SUMMARY OF RESULT OF A FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE*

Day p.i.	liver	lung	small intestine	blood	spleen	kidney	brain
3	322	1	0	0	0	0	
5	887	0	0	0	0	0	
7	0	293	0	0	0	0	0
14	0	0	0	0	0	0	0
total	1209	294	0	0	0	0	0

* Data express addition of 5 animals

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2.1**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 1 p.i. (number of larvae collected)

	1*	2	3	4	5	total
Liver	7	5	5	0	4	21
Lungs	10	0	0	0	0	10
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	17	5	5	0	4	31

* Individual animals

Table 2.2**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 2 p.i. (number of larvae collected)						
	6*	7	8	9	10	total
Liver	35	70	141	105	25	376
Lungs	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	35	70	141	105	25	376

* Individual animals

Table 2.3**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 3 p.i. (number of larvae collected)

	11*	12	13	14	15	total
Liver	90	78	10	105	122	405
Lungs	0	6	0	0	0	6
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	90	84	10	105	122	411

* Individual animals

Table 2.4**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 5 p.i. (number of larvae collected)

	16*	17	18	19	20	total
Liver	39	67	56	90	45	297
Lungs	0	0	0	5	0	5
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	39	67	56	95	45	302

* Individual animals

Table 2.5**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 6 p.i. (number of larvae collected)

	21*	22	23	24	25	total
Liver	4	5	4	4	8	25
Lungs	9	8	0	6	7	30
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	13	13	4	10	15	55

* Individual animals

Table 2.6**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 7 p.i. (number of larvae collected)

	26*	27	28	29	30	total
Liver	0	0	0	0	0	0
Lungs	22	7	17	28	18	92
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	22	7	17	28	18	92

* Individual animals

Table 2.7**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 8 p.i. (number of larvae collected)						
	31*	31	33	34	35	total
Liver	6	0	6	0	0	12
Lungs	7	11	13	11	30	72
Small intestine	0	2	1	2	1	6
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	13	13	20	13	31	90

* Individual animals

Table 2.8**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE
OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day 9 p.i. (number of larvae collected)						
	36*	37	38	39	40	total
Liver	0	0	0	0	0	0
Lungs	10	15	0	19	6	50
Small intestine	0	0	0	0	0	0
blood	0	0	0	0	0	0
spleen	0	0	0	0	0	0
kidneys	0	0	0	0	0	0
brain	0	0	0	0	0	0
Total	10	15	0	19	6	50

* Individual animals

Table 2.9**FOLLOW-UP STUDY OF THE COURSE OF MIGRATING LARVAE OF ASCARIS SUUM IN EXPERIMENTALLY INFECTED MICE**

Day p.i.	liver	lung	small intestine	blood	spleen	kidney	brain
1	21	10	0	0	0	0	
2	376	0	0	0	0	0	
3	405	6	0	0	0	0	
5	297	5	0	0	0	0	
6	25	30	0	0	0	0	0
7	0	92	0	0	0	0	0
8	12	72	6	0	0	0	0
9	0	50	0	0	0	0	0

Dose of infection 2000 embryonated eggs per mouse

The total number of larvae recovered 1,76%

* Data express addition of 5 animals

Table 3

RECOVERY OF MIGRATING A.SUUM LARVAE FROM THE LIVERS OF MICE IMMUNISED WITH VARIOUS ASCARIS ANTIGENS THREE DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGG (EXPERIMENT II)

Group (n=5)	Immunised with	Number of larvae recovered					P-value (Wilcoxon) vs Group V	
		individual values			mean per mouse			
I	L2 somatic	28	24	30	8	0	18	0.01
II	L3/4 somatic	20	22	4	14	26	17.2	0.01
III	ES/L2	36	4	28	0	0	13.6	0.01
IV	ES/L3/4	32	52	4	0	7	19	0.05
V	non-treated	90	193	88	119	32	105.4	

vs = versus

Table 4

RECOVERY OF MIGRATING A.SUUM LARVAE FROM THE LUNGS OF MICE IMMUNISED WITH VARIOUS ASCARIS ANTIGENS SEVEN DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGG (EXPERIMENT II)

Group (n=5)	Immunised with	Number of larvae recovered					P-value (Wilcoxon) vs Group V	
		individual values			mean per mouse			
I	L2 somatic	5	7	7	6	6	6.2	n.s.
II	L3/4 somatic	0	0	6	0	0	1.2	n.s.
III	ES/L2	6	0	0	11	1	3.6	n.s.
IV	ES/L3/4	2	1	1	0	1	1.0	n.s.
V	non-treated	1	2	15	2	5	5.0	

vs = versus

n.s. = not significant

Table 5

RECOVERY OF MIGRATING A.SUUM LARVAE FROM THE LIVER OF MICE IMMUNISED WITH VARIOUS ASCARIS ANTIGENS THREE DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGG (EXPERIMENT III)

Group (n=5)	Immunised with	Number of larvae recovered					P-value (Wilcoxon) vs Group V		
		individual values					mean per mouse	vs Group IV	vs Group V
I	ES/L2 A.suum	0	0	0	11	18	5.8	n.s.	n.s.
II	ES/L3/4 A.suum	0	0	0	0	22	4.4	n.s.	n.s.
III	ES/L2 + ES/L3/4 A.suum	0	0	0	0	13	2.6	n.s.	n.s.
IV	saline adjuvant	0	63	65	76	92	59.2	n.s.	n.s.
V	non-treated	0	33	69	84	97	56.6		

vs = versus

n.s. = not significant

Table 6

RECOVERY OF MIGRATING A.SUUM LARVAE FROM THE LUNGS OF MICE IMMUNISED WITH ES-ANTIGENS SEVEN DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGG (EXPERIMENT III)

Group (n=5)	Immunised with	Number of larvae recovered					P-value (Wilcoxon) vs Group V		
		individual values					mean per mouse	vs Group IV	vs Group V.
I	ES/L2 A.suum	0	0	8	0	0	1.6	<0.05	<0.05
II	ES/L3/4 A.suum	7	0	0	4	0	2.2	<0.05	<0.05
III	ES/L2 + ES/L3/4 A.suum	6	0	9	0	0	3.0	<0.05	<0.05
IV	saline adjuvant	34	25	8	28	19	22.8		
V	non-treated	29	24	36	19	22	26.0		

vs = versus

Table 7

RESULT OF SEROLOGICAL EXAMINATIONS (BY ELISA) ON SERA FROM MICE IMMUNISED WITH VARIOUS ES ANTIGENS OF ASCARIS SUUM LARVAE AND CONTROL ANIMALS (EXPERIMENT III) AT DAY 45 AND 49

Ascaris antigens used in ELISA	Reciprocal ELISA antibody titers in pooled sera from mice (n=5) immunised with:						
	ES/L2		ES/L3/4		ES/L2 + ES/L3/4		FCA + FIA
	Day 45	Day 49	Day 45	Day 49	Day 45	Day 49	Day 45 & 49
ES/L2	320	320	<20	40	160	160	<20
ES/L3/4	<20	<20	80	80	20	40	<20
Somatic L2	160	80	<20	40	160	160	<20
Somatic L3/4	160	320	40	160	80	160	<20

Table 8

RECOVERY OF MIGRATING A.SUUM AND A.LUMBRICOIDES LARVAE FROM THE LIVER OF MICE IMMUNISED WITH ES ANTIGENS OF LARVAE EITHER SPECIES THREE DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGGS (EXPERIMENT IV)

Group (n=5)	Immunised with	Challenged with	Number of larvae recovered					P-value (Wilcoxon) vs Group V	
			individual values						mean per mouse
I	A.suum	A.suum	0	5	5	9	12	6.2	vs IV<0.05 vs VI<0.05
II	A.suum	A.lumbricoides	0	3	4	6	8	4.2	vs V<0.05 vs VII<0.05
III	A.lumbricoides	A.lumbricoides	0	2	3	4	6	3.0	vs V<0.05 vs VII<0.05
IV	saline/adjuvant	A.suum	26	27	29	37	84	40.6	
V	saline/adjuvant	A.lumbricoides	19	37	41	53	67	43.4	
VI	non-treated	A.suum	36	39	42	51	58	45.2	
VII	non-treated	A.lumbricoides	24	28	33	42	54	36.2	

vs = versus

Table 9

RECOVERY OF MIGRATING A.SUUM AND A.LUMBRICOIDES LARVAE FROM THE LUNGS OF MICE IMMUNISED WITH ES ANTIGENS OF LARVAE EITHER SPECIES SEVEN DAYS AFTER ORAL CHALLENGE WITH 2,000 EMBRYONATED EGGS (EXPERIMENT IV)

Group (n=5)	Immunised with	Challenged with	Number of larvae recovered					P-value (Wilcoxon) vs Group V	
			individual values						mean per mouse
I	A.suum	A.suum	4	6	0	0	8	3.6	vs IV<n.s vs VI<n.s
II	A.suum	A.lumbricoides	0	3	0	2	0	1.0	vs V<n.s vs VII<n.s
III	A.lumbricoides	A.lumbricoides	0	0	3	0	0	0.6	vs V<n.s vs VII<n.s
IV	saline/adjuvant	A.suum	28	32	19	0	37	23.2	
V	saline/adjuvant	A.lumbricoides	0	15	10	16	8	9.8	
VI	non-treated	A.suum	30	18	25	14	23	22.0	
VII	non-treated	A.lumbricoides	20	8	19	0	12	11.8	

vs = versus

n.s = not significant

Table 10

RESULTS OF SEROLOGICAL EXAMINATION (BY ELISA) ON SERA FROM MICE IMMUNISED WITH ES/L2 ANTIGENS OF A.SUUM AND A.LUMBRICOIDES RESPECTIVELY AND CONTROL ANIMALS AFTER ORAL CHALLENGE INFECTION WITH EMBRYONATED EGGS OF A.SUUM AND A.LUMBRICOIDES AT DAY 45 AND 49 (EXPERIMENT IV)

Reciprocal ELISA natibody titers in pooled sera (n=5) from mice immunised with:							
Ascaris antigens used in ELISA	ES/L2 A.s*		ES/L2 A.s**		ES/L2 A.1**		Control
	Day 45	Day 49	Day 45	Day 49	Day 45	Day 49	Day 45 and Day 49
ES/L2 A.s	80	80	80	80	160	160	<20
ES/L2 A.1	20	<20	20	20	160	160	<20
ES/L3/4 A.s	20	<20	20	20	<20	<20	<20
somatic L2 A.s	40	40	40	40	80	40	<20
Somatic L3/4 A.s	40	40	40	40	20	20	<20

* challenged with A.suum

** challenged with A.lumbricoides

A.s: Ascaris suum

A.1: Ascaris lumbricoides

Table 11

RECOGNITION OF ES ANTIGENS OF ASARIS SUUM AND ASCARIS LUMBRICOIDES BY WESTERN BLOTTING AFTER A CROSS IMMUNISATION EXPERIMENTS

Mouse antisera against	Ascaris antigens	ES/L2 <u>A.suum</u>	ES/L3,4 <u>A.suum</u>	ES/L2 <u>A.lumbricoides</u>
ES/L2	<-- A.suum	2 bands	-	-
ES/L3,4	<-- A.suum	-	1 band	-
ES/L2	<-- A.lumbricoides	1 band	1 band	1 band

Table 12

SOMMARY OF THE NUMBER OF MICE FULLY PROTECTED (NO MIGRATING LARVAE DETECTED) IN EXPERIMENT AND CONTROL GROUPS

	Protected mice	None protected mice	
E	49	61	110
C	7	93	100
	56	154	210

$p < 0.001$

Figure 1

LIFE-CYCLE OF ASCARIS LUMBRICOIDES

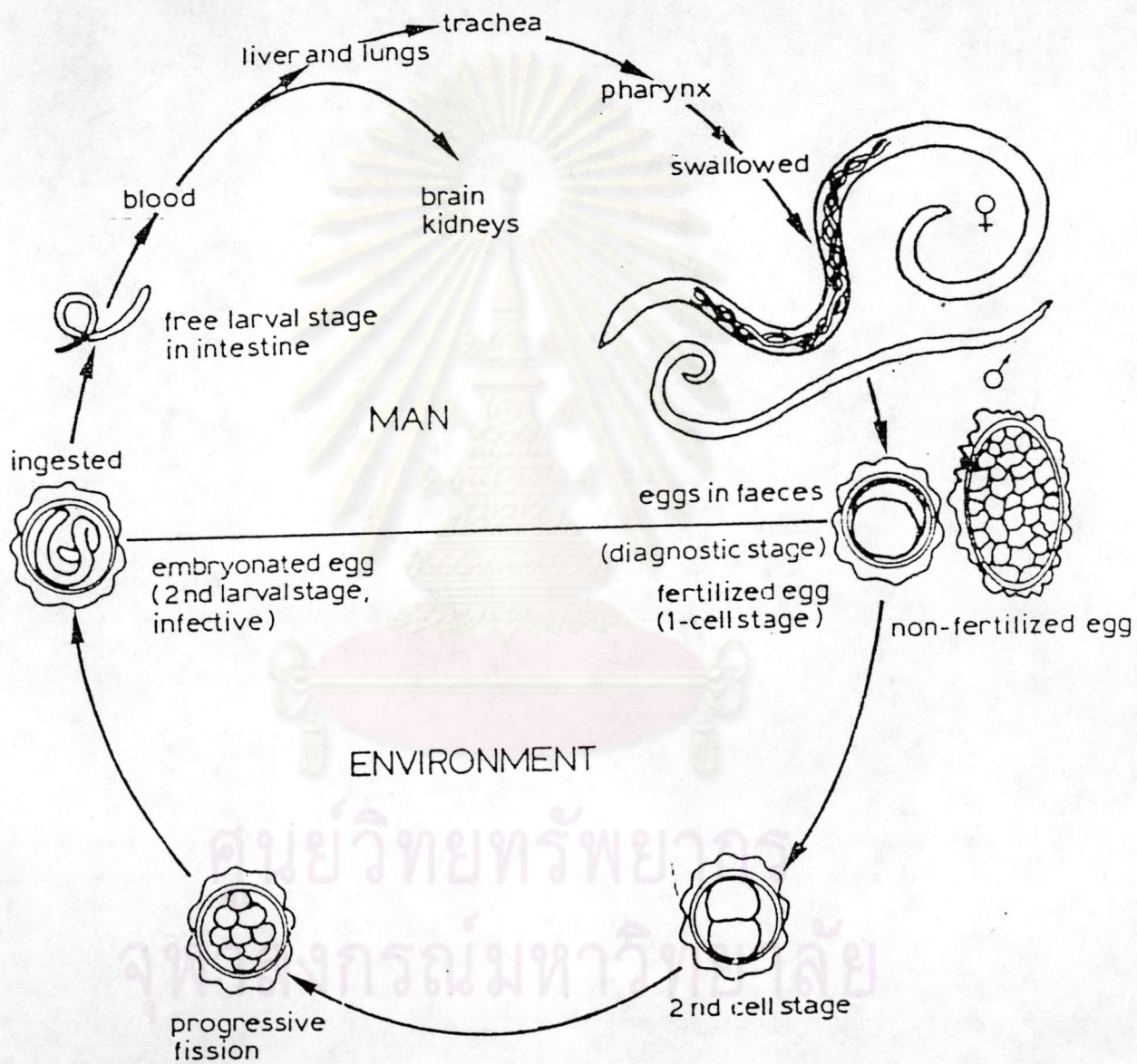


Figure 2

RECOVERY OF MIGRATING ASCARIS SUUM LARVAE FROM ORGANS OF MICE INFECTED WITH 2000 EMBRYONATED EGGS

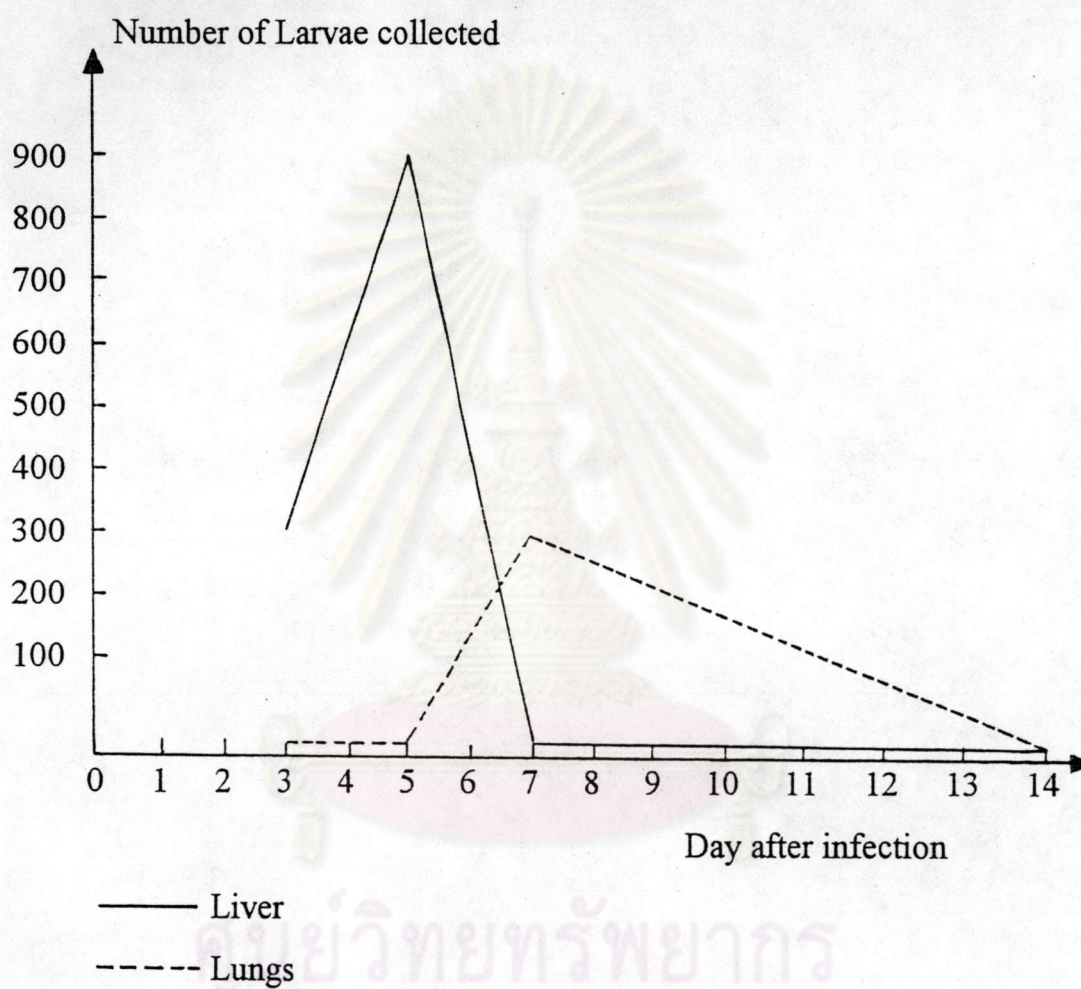


Figure 3

RECOVERY OF MIGRATING ASCARIS SUUM LARVAE FROM ORGANS OF MICE INFECTED WITH 2000 EMBRYONATED EGGS.

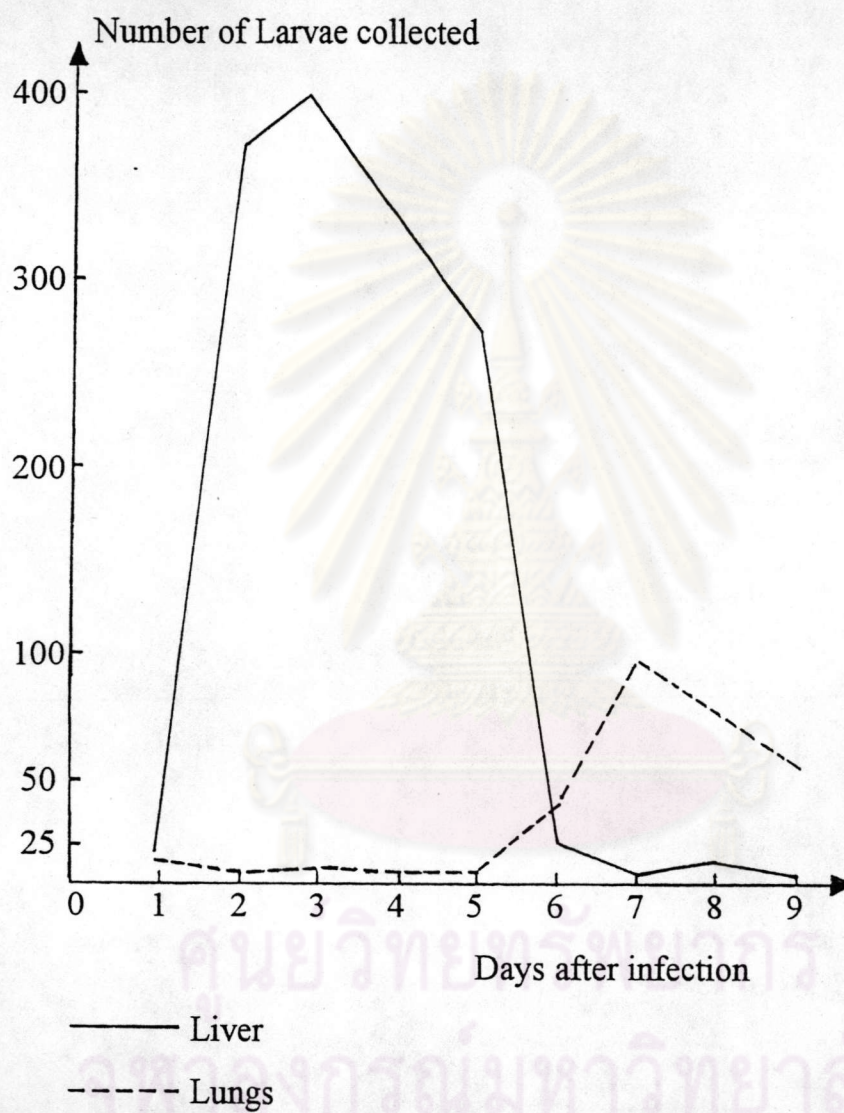
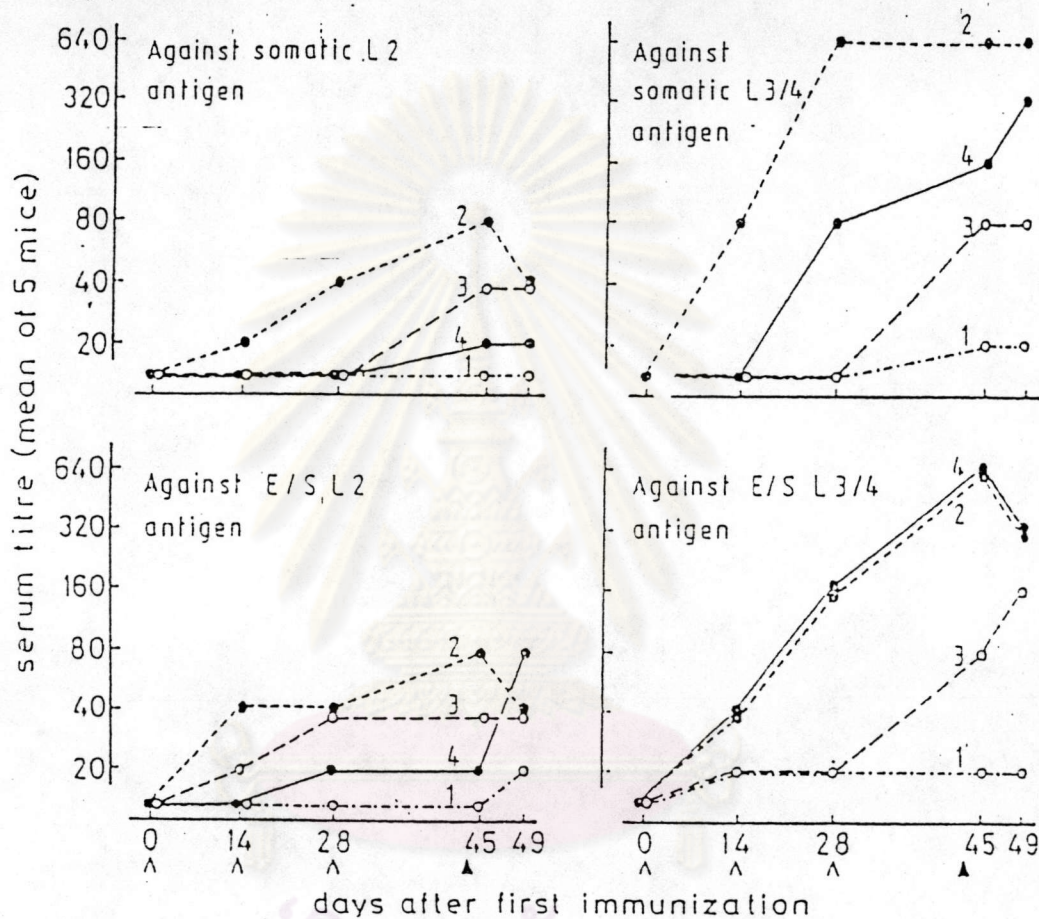


Figure 4

ELISA TITRES IN SERA OF GROUPS OF MICE KILLED AT VARIOUS DAYS AFTER IMMUNIZATION WITH A.SUUM LARVAL ANTIGENS.



Antigens used for immunization:

- 1 ○---○ somatic L2
- 2 ●---● " L3/4
- 3 ○---○ E/S L2
- 4 ●---● " L3/4

△: day of immunization

▲: day of challenge with 2,000 embryonated *A. suum* eggs

Figure 5

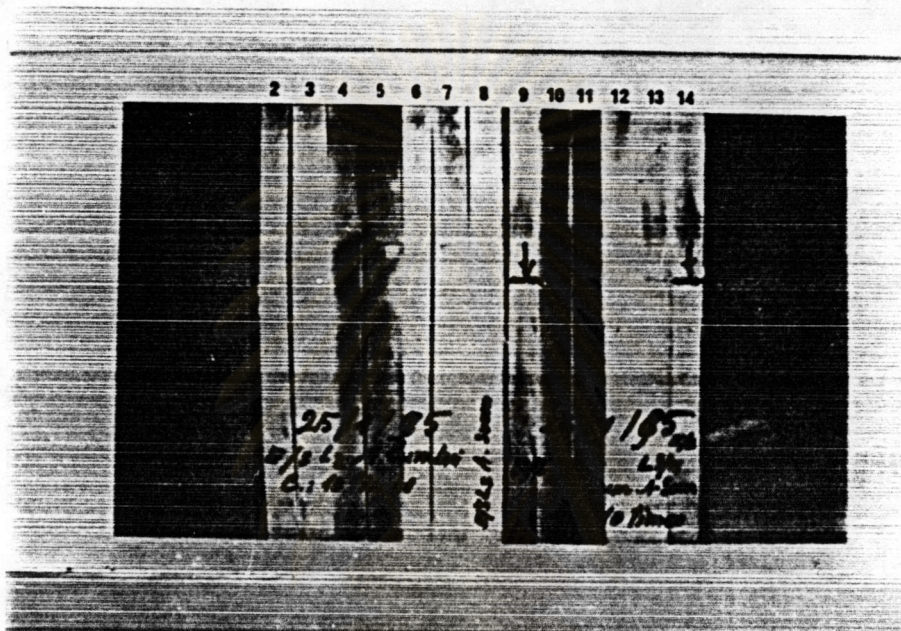
SDS-PAGE OF ES ANTIGENS OF L2 LARVAE OF ASCARIS SUUM AND IMMUNOBLOTTING WITH IMMUNESERA OF MICE TREATED WITH ES ANTIGENS OF L2 AND L3/4 LARVAE RESPECTIVELY.

**Antigens and antisera applied:**

- lane 1: ES/L2 A.suum, 10 times concentrated: immuneserum day 45 against ES/L2 1 : 10 dilution
- lane 2: ES/L2 A.suum, 10 times concentrated: against ES/L2 1 : 25 dilution
- lane 3: ES/L2 A.suum, 10 times concentrated; immuneserum day 45, against ES/L3/4 1 : 10 dilution
- lane 4: ES/L2 A.suum, 10 times concentrated; against es/l3/4 1 : 25 dilution
- lane 5: ES/L3/4 A.suum, 10 times concentrated: immuneserum against ES/L2 day 45, 1 : 10 dilution
- lane 6: ES/L3/4 A.suum, 10 times concentrated; against ES/L2, 1 : 25 dilution
- lane 7: ES/L3/4 A.suum, 10 times concentrated, immunserum against ES/L3/4, day 45, 1 : 10 dilution
- lane 8: ES/L3/4 A.suum, 10 times concentrated, against ES/L3/4, 1 : 25 dilution

Figure 6

SDS-PAGE OF ES ANTIGENS OF L2 AND L3/4 LARVAE OF ASCARIS SUUM AND L2 LARVAE OF A.LUMBRICOIDES FOLLOWED BY IMMUNOBLOTTING USING VARIOUS IMMUNESERA FROM MICE

**Antigens applied:**

lane 2-7: ES/L2 A.lumbricoides concentrated 10 x

lane 8-12: ES/L2 A.suum concentrated 10 x

lane 13-14: ES/L3/4 A.suum concentrated 10 x

Immunesera applied against:

ES/L2 A.lumbricoides day 45 dilution 1 : 25, lanes 2, 8, 13

ES/L2 A.lumbricoides day 49 dilution 1 : 25, lanes 3, 9, 14

ES/L2 A.suum day 45 dilution 1 : 25, lanes 4, 10

ES/L2 A.suum day 49 dilution 1 : 25, lanes 5, 11

ES/L3/4 A.suum day 45 dilution 1 : 25, lanes 6, 12

ES/L3/4 A.suum day 49 dilution 1 : 25, lane 7

Figure 7

SDS-PAGE OF ES ANTIGEN OF L2 LARVAE OF ASCARIS LUMBRICOIDES FOLLOWED BY IMMUNOBLOTTING USING VARIOUS IMMUNESERA FROM MICE

**Antigens applied:**

ES/L2 *A.lumbricoides*, concentrated 20 times in all lanes

Imunesera applied against:

- lane 1: ES/L2 *A.lumbricoides*, day 45, dilution 1 : 10
- lane 2: ES/L2 *A.lumbricoides*, day 45, dilution 1 : 25
- lane 3: ES/L2 *A.lumbricoides*, day 40, dilution 1 : 10
- lane 4: ES/L2 *A.lumbricoides*, day 49, dilution 1 : 25
- lane 5: ES/L2 *A.suum*, day 49, dilution 1 : 10
- lane 6: ES/L3/4 *A.suum*, day 49, dilution 1 : 10