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Visceral Larva Migrans in Mice Caused by Eating *Toxocara canis*-Infected Chick Livers*

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Abstract: In this study, two subjects, 1) whether Visceral Larva Migrans (VLM) takes place when *T. canis* larvae-infected chick livers are eaten and 2) whether any differences are present between larval distributions, clinical signs and pathological disorders of the infections caused by eggs and infected liver, were investigated in the chick-mouse model. For this purpose, 15-day-old broiler chicks (n=42) and 3-month-old albino mice (n=62) were used. Thirty chicks were each infected orally with 5000 infective *T.canis* eggs. These chicks, composed of 6 groups, were necropsied at 2-day intervals and their livers were fed to 42 mice in 6 experimental groups. In another group, experimental mice were inoculated with 1250 (2 mice), 2500 (2 mice) and 5000 (2 mice) infective eggs of *T. canis*. Two non-infected mice for each experimental group were kept as control. All mice were necropsied on day 6 and they were examined for *T.canis* larvae. The control mice presented neither behavioral disorders nor pathological changes and presented no larvae. Nine experimental mice died before the necropsy day. *Toxocara canis*-larvae were recovered from all experimental mice except one because this mouse was completely eaten by other mice. It was observed that the mice fed on infected liver were more affected by infection than those inoculated with the eggs. It is concluded that the consumption of *T. canis* infected raw poultry liver leads to toxocariosis in people.

Key Words: Visceral larva migrans, *Toxocara canis*, mice, chicken, zoonosis

Farelerde, *Toxocara canis*'le Enfekte Civciv Karaciğeri Yemeyle Viseral Larva Migrans

Özet: Bu çalışmada 1) *T.canis* larvasıyla enfekte civciv karaciğeri yenilerek VLM (Viseral Larva Migrans) oluşup oluşmayacağı ve 2) yumurta ve enfekte karaciğerle oluşturulan enfeksiyonlarda larva dağılımı, klinik belirtiler ve patolojik bozukluklar arasında herhangi bir farklılığın olup olmadığı civciv-fare modeli içinde araştırıldı. Çalışmada 15 günlük 42 broiler civciv ve 3 aylık 62 albino fare kullanıldı. Civcivlerden 30 tanesi 5000'er adet enfektif *T.canis* yumurtası ile oral yolla enfekte edilmiştir. Diğer civcivler, kontrol fareleri için enfekte edilmeden bırakılmıştır. Civcivler 2'şer gün arayla 6 grup halinde açılmış ve her grubun karaciğerleri aynı gruptaki farelere yedirilmiştir. Ayrıca diğer bir deney grubunda oral yolla 2 fareye 1250, 2 fareye 2500 ve 2 fareye 5000 adet enfektif *T.canis* yumurtası verilmiştir. Tüm enfeksiyon grupları için 2'şer adet fare kontrol olarak bırakılmıştır. Farelerin nekropsileri enfeksiyondan sonraki 6. günde yapılmış ve fareler *T.canis* larvaları yönünden muayene edilmiştir. Kontrol gruplarındaki farelerde herhangi bir davranış bozukluğu oluşmamış, organlarında patolojik değişikliğe ve larvaya rastlanmamıştır. Enfekte edilen farelerden 9 tanesi nekropsi gününden önce ölmüş, bir tanesi diğerleri tarafından tamamen yenmiştir. Enfekte edilen tüm farelerden diğerleri tarafından tamamen yenilen biri hariç diğerlerinde *T.canis* larvaları görülmüştür. Enfeksiyondan, enfekte karaciğer yiyen farelerin, yumurta ile enfekte edilenlerden daha fazla etkilendiği gözlenmiştir. Ayrıca, enfekte karaciğer yiyen farelerin akciğerlerinden larva geri elde etme oranlarının daha yüksek olduğu görülmüştür. Çalışmada *T.canis*'le enfekte çiğ kanatlı karaciğeri yenmesinin insanlarda toxocariosis'e yol açacağı sonucuna varılmıştır.

Anahtar Sözcükler: Visceral larva migrans, *Toxocara canis*, fare, tavuk, zoonosis

Introduction

It is well known that ingestion of infective *Toxocara canis* eggs leads to Visceral Larva Migrans (VLM) in poultry and mammals including man (1). Poultry can gain the VLM infection from an unsanitary environment contaminated with *T. canis* eggs and/or infected earthworms (2). It is doubtless that such an environment is of importance to wild poultry and to domestic ones reared outside of a poultry house. In poultry, liver is an important organ in which *T.canis* larvae accumulate in the greatest number (3-8). The parasite may cause severe disorders in man (9) and there are some cases of human toxocariosis related to eating raw poultry, raw poultry liver and raw cow liver in Japan (10-12). The experimental infection of mice was performed by oral inoculation of larvae obtained from Japanese quails (7)

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and chickens (5). However, to the best of our knowledge, this subject has not yet been confirmed on a natural model involving the eating of infected organs. Min (13) suggested that larvae transmitted from another paratenic host were more pathogenic.

In this study, two subjects, 1) whether VLM takes place when *T.canis* larvae-infected chick livers are eaten and 2) whether any differences are present between larval distributions, clinical signs and pathological disorders of the infections caused by eggs and infected liver, were investigated in the chick-mouse model.

Materials and Methods

Sixty-two albino mice aged 3 months and 42 broiler chicks aged 15 days were used in the study. One egg (Egg) and 6 liver (L1-L6) groups were established. Each group was comprised of experimental and control subgroups. The study design is given in Table 1.

The eggs of *T. canis* were obtained from the uteri of female parasites and developed to the infective stage in 1% formalin saline (6,14). The infective eggs in 1% formalin-saline were kept at 4ºC until used. The eggs were given to the chicks and the mice orally. The chicks were necropsied between 2 and 12 days after inoculation, with a two-day interval according to the groups. The livers obtained from the chicks were fed to the mice in the same subgroup.

All mice were necropsied on day 6. At necropsy, gross pathological disorders were recorded. The liver, lungs, brain and carcass of all mice were examined for the presence of *T. canis* larvae and larval counting was performed. For this purpose, the digestion technique (15) was used for the livers, lungs and carcasses and squash preparations for the brain.

Results

Larval recovery results:

In control mice, no larvae were found. But, *Toxocara canis* larvae were recovered from all experimental mice except one in group L6, because this mouse had died on day 3 and had been completely eaten by other mice. The number of larvae recovered is given in Table 2 and 3, and the comparison of the larval accumulations in the organs is demonstrated in the Figure.

Behavioral and pathological findings:

In the control mice: Neither behavioral disorders nor pathological changes were observed.

In the experimental mice of the egg group: The mice infected with 2500 and 5000 eggs were dull. At necropsy, petechial hemorrhages were observed on the surfaces of the lungs and brains of all infected mice in the egg group. Similar hemorrhages were also seen on the surface of the livers of the mice infected with 2500 and 5000 eggs. The animals infected with 5000 eggs died on day 3.

Table 1. The Study Design.

Group	Subgroup.	NM	NEM	NDM	Group.	Subgroup.	NC	NEC	NDC	NM	NDM
Egg	Exp.	2	5000	6	L1	Exp.	5	5000	2	5	6
		2	2500	6		Contr.	2	0	2	2	6
		2	1250	6	L2	Exp.	5	5000	4	5	6
	Contr.	2	0	6		Contr.	2	0	4	2	6
					L3	Exp.	5	5000	6	8	6
						Contr.	2	0	6	2	6
					L4	Exp.	5	5000	8	8	6
						Contr.	2	0	8	2	6
					L5	Exp.	5	5000	10	8	6
						Contr.	2	0	10	2	6
					L6	Exp.	5	5000	12	8	6
						Contr.	2	0	12	2	6

Abr.: Number of mice (NM) and chicks (NC). Number of eggs given to each mouse (NEM) and to each chick (NEC). Necropsy days of mice (NDM) and chicks (NDC)

Table 2. The Larval Recovery Results from the Experimental Mice Necropsied on Day 6.

Gr: Egg (Eggs inoculated)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1a	died	on	day	3					
2a	died	on	day	3					
3b	27	7.54	17	4.75	146	40.8	168	46.9	358
4b	18	4.71	22	5.76	118	30.9	224	58.6	382
5c	51	14.7	51	14.7	100	28.9	144	41.6	346
6c	25	10.1	21	8.47	69	27.8	133	53.6	248
Aver	30.3	9.07	27.8	8.32	108	32.5	167	50.1	334

Gr: L1 (fed on livers of chicks killed 2 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	52	20.0	120	46.2	88	33.8	0	0.0	260
2	40	17.7	96	42.5	90	39.8	0	0.0	226
3	50	11.6	248	57.7	132	30.7	0	0.0	430
4	99	35.6	88	31.7	87	31.3	4	1.44	278
5	8	8.79	25	27.5	58	63.7	0	0.0	91
Aver	49.8	19.4	115	44.9	91.0	35.4	0.80	0.311	257

Gr: L2 (fed on livers of chicks killed 4 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	died	on	day	2					
2	died	on	day	2					
3	died	on	day	5					
4	24	13.7	116	66.3	27	15.4	8	4.57	175
5	32	8.74	207	56.6	127	34.7	0	0.0	366
Aver	28.0	10.4	162	59.7	77.0	28.5	4.0	1.48	271

Gr: L3 (fed on livers of chicks killed 6 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	34	7.83	94	21.7	106	24.4	200	46.1	434
2	9	2.88	150	48.1	43	13.8	110	35.3	312
3	7	3.72	22	11.7	39	20.7	120	63.8	188
4	9	4.57	12	6.09	28	14.2	148	75.1	197
5	7	6.03	9	7.76	16	13.8	84	72.4	116
6	9	30.0	20	66.7	1	3.33	0	0.0	30
7	11	33.3	15	45.5	7	21.2	0	0.0	33
8	4	1.61	29	11.7	35	14.1	180	72.6	248
Aver	11.3	5.78	43.9	22.5	34.4	17.7	105	54.0	195

Gr: L4 (fed on livers of chicks killed 8 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	died	on	day	4					
2	23	6.15	165	44.1	78	20.9	108	28.9	374
3	43	13.0	124	37.6	75	22.7	88	26.7	330
4	6	1.87	205	63.9	64	19.9	46	14.3	321
5	7	2.88	79	32.5	56	23.0	101	41.6	243
6	26	9.52	73	26.7	37	13.6	137	50.2	273
7	48	11.5	233	55.6	53	12.6	85	20.3	419
8	30	6.42	343	73.4	43	9.21	51	10.9	467
Aver	26.1	7.54	175	50.4	58.0	16.7	88.0	25.4	347

Gr: L5 (fed on livers of chicks killed 10 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	died	on	day	5					
2	died	on	day	5					
3	39	7.75	304	60.4	64	12.7	96	19.1	503
4	48	8.44	354	62.2	95	16.7	72	12.7	569
5	54	12.9	255	60.9	57	13.6	53	12.6	419
6	33	7.84	152	36.1	66	15.7	170	40.4	421
7	45	16.7	106	39.4	56	20.8	62	23.0	269
8	33	4.12	603	75.3	63	7.87	102	12.7	801
Aver	42.0	8.45	296	59.5	66.8	13.4	92.5	18.6	497

Gr: L6 (fed on livers of chicks killed 12 days later)

No	Li	%	Lu	%	Br	%	Ca	%	Tot
1	died	on	day	3					
2	48	10.6	179	39.5	109	24.1	117	25.8	453
3	20	8.10	104	42.1	67	27.1	56	22.7	247
4	6	3.59	47	28.1	37	22.2	77	46.1	167
5	76	9.29	457	55.9	129	15.8	156	19.1	818
6	46	13.7	104	31.0	96	28.6	90	26.8	336
7	46	18.9	64	26.2	70	28.7	64	26.2	244
8	62	17.2	165	45.7	77	21.3	57	15.8	361
Aver	43.4	11.6	160	42.7	83.6	22.3	88.1	23.5	375

Mouse infected with 5000 (a), 2500 (b), 1250 (c) eggs, Li: Liver, Lu: Lungs, Br: Brain, Ca: Carcass, Aver: Average

Group-No	DD	Li	%	Lu	%	Br	%	Ca	%	Tot
Egg-1	3	49	4.9	200	20	28	2.8	728	72	1005
Egg-2	3	78	8.9	93	11	66	7.5	640	73	877
L2-1	2	60	5.6	876	82	36	3.4	96	9	1068
L2-2	2	120	20	470	77	22	3.6	0	0	612
L2-3	5	28	5.9	324	68	125	26	0	0	477
L4-1	4	195	21	644	70	20	2.2	63	6.8	922
L5-1	5	72	?	504	?	ne	?	87	?	663
L5-2	5	69	6.3	860	79	63	5.8	95	8.7	1087
L6-1	3	ne	?	ne	?	ne	?	ne	?	?

DD: Death day, Li: Liver, Lu: Lungs, Br: Brain, Ca: Carcass, ne: not examined because subject was completely eaten by others

Table 3. The Larval Recovery Results of Experimental Mice Dying before the Necropsy on Day 6.

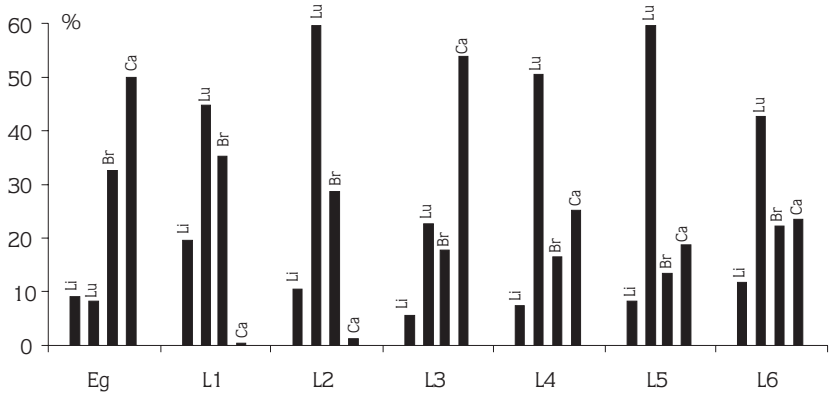

Figure. The Comparison of the Larval Accumulations in the Organs.

In the experimental mice of the liver groups: Within 24 hours after eating infected livers, all animals became dull and their coats were rough and matted. The experimental mice between the groups L3 and L6 had closed eyelids and showed incoordination two or three days after eating infected livers. At necropsy, hemorrhagic areas, irregular fine white stippling (additionally red stippling in those of group L2) were seen on the surface of the lungs of all experimental mice. In the experimental mice between the groups L3 and L6, there were hemorrhagic foci on the surfaces of the brains. After eating infected liver, 7 experimental mice died before necropsy day. These animals and their groups were as follows: In group L2, 2 animals on day 2 and 1 on day 5; in group L4, 1 on day 4; in group L5, 2 on day 5 (the brain of one was eaten); in group L6, 1 on day 3 (completely eaten).

Discussion

Seven patients with human toxocariosis having a history of ingesting raw poultry, raw poultry liver or raw cow liver were reported from Japan (10-12). Nagakura et al. (12) suggested that eating raw poultry or cow liver containing *T.canis* larvae might possibly cause toxocariosis in man. The experimental infection of mice was performed by oral inoculation of larvae obtained from Japanese quails (7) and chickens (5). It was suggested that the larvae transmitted from another paratenic host were more pathogenic (13).

In the present study, the mice were infected by naturally eating infected chick livers and it seemed that the mice fed on infected liver were more affected from infection than those inoculated with the eggs. The larval accumulation in organs was the other difference observed

in our study. The percentage rate of larvae recovered from the lungs of the mice fed on infected liver was much higher than in those of mice inoculated with eggs.

It is concluded that the consumption of *T.canis* infected raw poultry liver leads to toxocariosis in people. Therefore, people must be aware of the danger of consuming raw liver especially from domestic poultry reared outside of the poultry house or from wild ones.

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