WHO Recommended Pre-exposure Prophylaxis for Rabies Using Japanese Rabies Vaccine

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After severe exposure to suspected rabid animal, WHO recommends a complete vaccine series using a potent effective vaccine that meets WHO criteria, and administration of rabies immunoglobulin (RIG). RIG is not available globally, and is not marketed in Japan. If pre-exposure prophylaxis for rabies is given, RIG is unnecessary even after severe exposure. It is thus important to give pre-exposure prophylaxis for rabies to people who plan to go to rabies-endemic areas.

In Japan, pre-exposure prophylaxis for rabies consists of 3 doses of cell-culture rabies vaccine. The first two doses are given 4 weeks apart, and the third dose is given 6-12 months after the first dose, all of which are injected subcutaneously (standard regimen). People who plan to travel abroad to rabies-endemic areas may know of their destinations only 1 or 2 months in advance at best. Therefore, it is virtually impossible to complete the 3 dose regimen for rabies in Japan.

Pre-exposure prophylaxis recommended by WHO consists of 3 doses given intramuscularly on days 0, 7, and 28, making it possible to complete pre-exposure prophylaxis in one month. This WHO recommended pre-exposure prophylaxis using Japanese cell-cultured rabies vaccine (PCEC-K) has not been studied, so we elected to fill the gap using PCEC-K, administered based on the WHO recommendation and examined its efficacy and safety.

Subjects were 26 healthy volunteers with no previous rabies vaccination giving oral and written consent. Vaccine was administered on days 0, 7, and 28, and rabies antibody levels were tested on days 7, 28, and 42. On day 7, every antibody level was negative. On day 28, antibody levels were between 0.7-3.5EU/mL, with the exception of 3 cases still negative. On day 42, all cases, including the 3 negative cases, exceeded 1.6EU/mL, providing sufficient protection against rabies. This result was not inferior compared to the standard regimen. Local adverse effects such as erythema and pain were noted, but none were serious.

In conclusion, WHO recommended pre-exposure prophylaxis for rabies using PCEC-K is considered effective and safe.

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Prolactin evokes lactational transmission of larvae in mice infected with *Toxocara canis*

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ABSTRACT

We investigated the trans-lactational maternal–neonatal transmission of *Toxocara canis* larvae in mice, with particular interest in the role of prolactin in their migration to the mammary gland. Two female mice were infected with 300 *T. canis* eggs soon after delivery of 27 offspring. After 1 week of breast-feeding, seven larvae were recovered from 4 of 13 offspring. After 2 weeks of lactation, 101 larvae were recovered from all the remaining offspring. Daily prolactin administration (5 μg) was performed 2 weeks before *T. canis* infection and continued until 2 weeks after infection in six non-pregnant female mice, which resulted in larval accumulation in the mammary gland. Furthermore, prolactin administration in female mice that had been infected with *T. canis* 4 weeks prior to prolactin treatment induced migration of larvae into the mammary gland. These findings suggest that prolactin is a promoting factor contributing to lactational transmission of *T. canis* larvae in mice.

1. Introduction

Human larval toxocariasis is a serious public health problem in many countries [1]. Adult worms of *Toxocara canis* parasitize the intestines of domestic dogs and wild carnivores, and the larval stage of the parasite opportunistically invades undefinitive hosts including humans, resulting in human larval toxocariasis [2]. The migration behavior of the larvae in undefinitive hosts has been well documented [3–5]. In mice, *T. canis* larvae begin to accumulate in the liver 2 days post-infection, and they continue to migrate via systemic circulation. Beyond the 10th day of infection, most have settled in the brain and muscle tissue [6–8]. The larvae found in skeletal muscle are encapsulated in granulomatous inflammatory tissue and can survive for a long period [4,8]; those in the brain tissue elicit minimal inflammatory response [4].

Furthermore, it has been established that trans-placental transmission is the major route for *T. canis* larvae migration from infected female dogs to puppies [9–13]. In mice, it has also been regarded that *T. canis* larvae are transmissible via placenta [14–16], although no previous studies demonstrated larvae from offspring. Recently, Reiterova et al. [17] observed that *T. canis* larvae in offspring from infected mother mice were recovered at the beginning of the 5th day post-delivery. Thus, lactational transmission rather than trans-placental migration was certainly a possible route of maternal–neonatal infection with *T. canis*. After infection, migrating larvae settle in skeletal muscle tissue, in which they are then arrested in granulomatous inflammatory tissue. A re-emergence mechanism for these arrested larvae during pregnancy, however, has yet to be identified. In the present study, we demonstrate that *T. canis* larvae are able to transmit from mother to neonate via the mammary gland, and that prolactin evokes lactational transmission of the arrested larvae.

2. Materials and methods

2.1. Animals

Conventional ICR mice and an inbred strain of BALB/c mice were purchased from CLEA Japan Inc., Tokyo. All experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

2.2. Infections

T. canis eggs were obtained from the uteri of adult worms collected from naturally infected puppies after the administration of anthelmintics. Mature embryonated eggs were prepared following the method of Ohsima [5], and 300 eggs were inoculated into each mouse via a Teflon tube with a siliconized glass syringe [18].

2.3. Recovery of larvae

Each of the mammary glands and whole body of newborn mice were digested with artificial gastric juice (0.5% of 1:10,000 pepsin and 0.7% hydrochloric acid, pH 1.5) for 3 to 4 h with vigorous agitation. After centrifugation, the larvae in the sediment were counted using a stereoscopic microscopy on a microscope slide (7 × 14 cm). Examination

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Table 1
Numbers of larvae recovered from neonates

Mother mouse	7th day after birth				14th say after birth			
	Number of neonates examined	Number of neonates larvae recovered	Number of larvae/neonate	Total number of larvae recovered	Number of neonates examined	Number of neonates larvae recovered	Number of larvae/neonate[a]	Total number of larvae recovered
#1	5	4	1.4±0.5 (1–3)[a]	7	6	6	9.5±0.8 (8–13)[a]	57
#2	8	0	0	0	8	8	5.5±0.9 (2–10)[a]	44

Neonates were allowed to breast-feed from the mother mice, which were infected with 300 eggs of *T. canis* immediately after delivery.
[a] Mean±SD (range).

of the brain was performed according to the method of Cho et al. [18]. In this experiment, we attempted to recover the larvae from skeletal muscle tissue by using the digestion method described above. However, the results were inconsistent in the number of larvae recovered from adult mice, because a large amount of sediments remained after digestion, making the counting of larvae using stereoscopic microscopy difficult. Therefore, we omitted the data on the muscle-stage larvae of the adult mice in this experiment.

2.4. Pathology of the mammary gland

Mammary glands of female mice were removed and fixed in 10% neutral formalin solution. Serial sections were then prepared and stained with haematoxylin and eosin. The degree of eosinophil infiltration around the mammary gland was estimated by the number of cells per square millimeter. We randomly selected 10 fields with a microscope of 100-fold magnification. To confirm cell identification, we observed at high magnification and counted the number of eosinophils. A careful attention was paid not to shift the original position.

2.5. Experimental design for trans-mammary transmission of larvae

Two pairs of 8-week-old ICR mice were mated in separate cages until the female mice became pregnant. Within 12 h after delivery, each of two female mice was infected with 300 eggs of *T. canis*, and then allowed to breast-feed their offspring for 2 weeks. The offspring were divided into two groups: one was killed on day 7 after delivery, the other was killed on day 14 after delivery. The number of larvae in the offspring was counted using the digestion method described above.

2.6. Effect of prolactin treatment in non-pregnant, infected mice

To investigate the effect of prolactin on the stimulation of larval migration from skeletal muscle or brain tissue, eight BALB/c female mice, at 8 weeks of age, were intraperitoneally injected with 5 μg of prolactin (100 mg/mL, Sigma, St. Louis, USA) in physiological saline everyday for 14 days, and were then infected with 300 *T. canis* eggs orally. Prolactin treatment was then continued for another 14 days. After treatment, the mammary glands were removed and the larvae were recovered. Two mice were used for histological purposes. As a control, seven additional mice were administered 0.5 mL of saline instead of prolactin.

Table 2
Effect of prolactin treatment in non-pregnant infected mice

Treatment	Number of mice used	Number of mice larvae identified in mammary glands	Number of mice larvae identified in the brain	Number of larvae in mammary glands of identified mice	Number of larvae in the brain of identified mice
Prolactin	6	6	6	9.8±3.5[a]	36±16.3[a]
Saline	5	0	5	0	34.4±24.2[a]

[a] Mean±SD.

2.7. Effect of prolactin treatment in chronically infected mice

Six BALB/c female mice, at 4 weeks of age, were infected with 300 *T. canis* eggs. Four weeks later, 5 μg of prolactin was intraperitoneally administered everyday for 14 days. The mammary glands were then examined as described above. As a control, equal numbers of BALB/c mice were employed, and 0.5 mL of saline was injected into the peritoneal cavity everyday for 14 days.

2.8. Statistics

Statistical analysis was performed using Student's *t* test. *P* values of <0.05 were considered statistically significant.

3. Results

3.1. Larval transmission to neonates via mammary gland after birth

Two mother mice delivered 11 and 16 offspring, respectively. The offspring from each infected mother mouse, which were infected with *T. canis* within 12 h after delivery, were randomly selected and sacrificed on day 7 or day 14 after delivery. Table 1 presents the number of offspring infected and the number of larvae recovered on each of these days. The rate of infection in the offspring and the average number of larvae recovered were higher in the group sacrificed on day 14 compared with that sacrificed on day 7. Additionally, the total number of larvae recovered was significantly higher in the day-14 group ($P<0.05$).

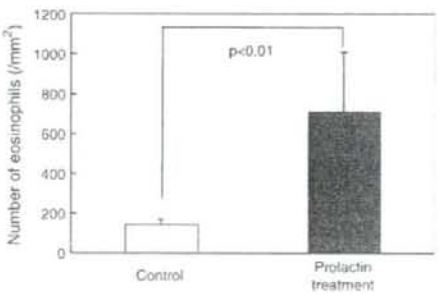

Fig. 1. Eosinophil counts around the capsules of mammary glands in mice. Solid bar, eosinophil count of prolactin-treated mice; open bar, that of untreated control mice. The mean number of eosinophils was 713.6±293.6 cells/mm² in the prolactin-treated group, and 144±21.3 cells/mm² in the saline-treated group. We randomly selected 10 fields with a microscope of 100-fold magnification. To confirm the cell identification, we observed at high magnification (×400) and counted the number of eosinophils. A careful attention was paid not to shift the original position.

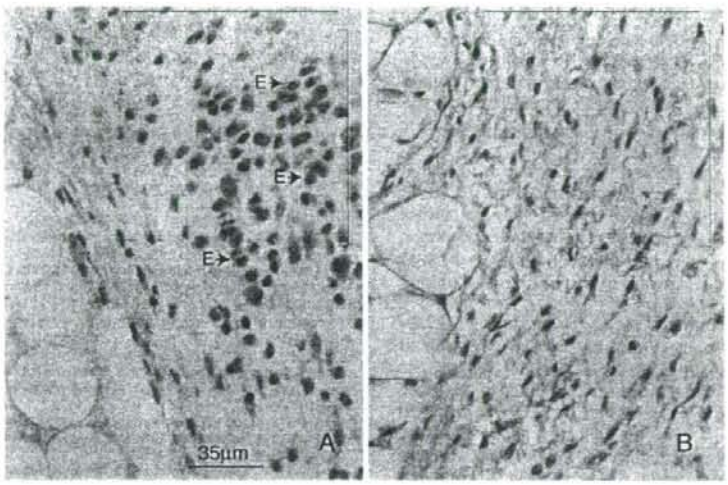

Fig. 2. Histopathological findings of mammary glands around the connective tissue in mice. Serial sections of mammary glands of female mice were stained with haematoxylin and eosin. Markedly higher eosinophilic (E) infiltrations around the connective tissue of the mammary gland were observed in the prolactin-treated mice (A) compared with the saline-treated mice (B).

3.2. Effect of prolactin on migration of larvae to the mammary gland

T. canis larvae were identified in the mammary glands of all infected mice, which were treated with 0.5 μg prolactin once a day intraperitoneally for 14 days before infection and 14 days after infection, although no larva was found in the control mice (Table 2). No significant difference in the number of larvae in the brain was observed between the prolactin-treated and saline-treated mice. These data suggest that prolactin might stimulate migration of larvae from skeletal muscle, the brain, or other organs to the mammary gland. Based on histological examination of 10 randomly selected fields, the eosinophil infiltrations around the capsule of the mammary gland were significantly increased in number in the prolactin-treated mice (713.6±293.6 6 cells/mm^2) compared with the saline-treated control mice (144±21.3 cells/mm^2, Figs. 1 and 2), suggesting that the inflammatory response against *T. canis* larvae was strong in the treated mice.

3.3. Effect of prolactin on chronically infected mice

Since administration of prolactin elicited a migration of larvae to the mammary gland, we next studied whether prolactin stimulates larval migration to the mammary glands from chronically infected mother mice in the absence of pregnancy. For this investigation, non-pregnant female mice, which had been infected with *T. canis* eggs 28 days previously, were administered prolactin for 14 days. Table 3 shows that larvae were recovered from the mammary glands in three of the four mice treated with prolactin, but no larva was found in the control mice. The number of eosinophils infiltrated in the mammary tissue was also significantly higher in the prolactin-treated group (Fig. 3).

In the prolactin-treated mice, glandular epithelial proliferation and dilatation of the ducts were observed, indicating a direct effect of prolactin against the mammary gland.

Table 3
Effect of prolactin treatment in chronically infected mice

Treatment	Number of mice used	Number of mice larvae identified in mammary glands	Number of mice larvae identified in the brain	Number of larvae in mammary glands of identified mice	Number of larvae in the brain of identified mice
Prolactin	4	3	4	3.8±1.9[a]	51.3±15.1[a]
Saline	4	0	4	0	49.8±5.7[a]

[a] Mean±SD.

4. Discussion

In this study, we demonstrate that *T. canis* larvae are able to migrate from the mother to neonates through suckling behavior, and that this migration can be induced by the administration of prolactin. While trans-placental migration of the larvae from female dogs to puppies has been established [9–13], few studies have investigated maternal–fetal transmission of the larvae in mice. Lee et al. [16] found that the larvae migrated in the uterus and placenta from the 9th day of pregnancy, and in the fetus from the 11th day of pregnancy when mother mice were infected during pregnancy. In addition, they

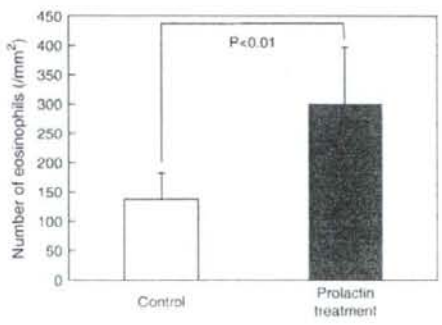

Fig. 3. Eosinophil counts around the capsules of mammary glands in chronically infected mice. Solid bar, eosinophil count of prolactin-treated mice; open bar, that of untreated control mice. The mean number of eosinophils was 300.8±95.6 cells/mm^2 in the prolactin-treated group, and 137.6±44.1 cells/mm^2 in the saline-treated group. Ten randomly selected fields at 100-fold magnification were observed via microscopy under a high magnification (400×).

identified larvae in the placenta and fetal blood vessels, histopathologically. They concluded that *T. canis* larvae were able to migrate through the placenta during pregnancy. However, because they did not examine the neonates after birth, they could not eliminate the possibility of trans-lactational transmission of the larvae from mother to neonates after delivery.

It is well documented that malaria infection induces placental injury, resulting in fetal loss in both humans and mice [19,20]. In murine toxocariasis, the litter sizes from infected mice are smaller than those from uninfected controls [21,22]. These data suggest that *T. canis* infection in mice can lead to mechanical injury of the placenta and a resultant decrease of litter size when the infection occurs during pregnancy.

Yet, in spite of these difficulties, newborns are still successfully delivered in most cases. In another previous study, larvae were found in offspring on day 5 after birth [15], suggesting suckling behavior might cause maternal–newborn transmission of *T. canis* larvae. In fact, our preliminary experiment revealed that larvae were first identified in offspring 11 days after birth (unpublished data). Thus, we hypothesized that larvae could migrate from mother to newborn mice through the mammary gland during suckling. The present findings support this hypothesis.

In general, *T. canis* larvae in mice settle in the brain and skeletal muscle after migration through the systemic circulation, and survive for a long period [4,8]. However, because we could not find any larvae in the mammary gland of non-pregnant infected mice, the larvae must be aroused by some sort of stimuli in order to migrate from those organs to the mammary gland. Prolactin, a lactogenic hormone, plays an essential role in the development of breast tissue. None of the non-pregnant mice not treated with prolactin showed the presence of larvae, in either the acute or chronic stage of infection, whereas prolactin-treated mice exhibited *T. canis* larvae infection in the mammary glands. One previous study discussed the relationship between *T. canis* infection and prolactin [23], reporting that the administration of prolactin led to a reduction in the number of larvae in infected mice. This may be related to the finding that prolactin acts as an immunomodulatory agent or proinflammatory cytokine in autoimmune diseases [24], and in several parasitic infections [25–28].

Eosinophil infiltration is a common feature in tissue-invading nematode infections, such as gnathostomiasis and trichinosis [29]. In toxocariasis, an eosinophilic granulomatous response is a typical pathological finding both in humans and in experimentally infected animals including mice [30,31]. Furthermore, eosinophil infiltration was demonstrated not only in the tissue adjacent to the larvae but also in that through which the larvae had passed [32]. These pathological changes are thought to be stimulated by the metabolic products from the larvae [29]. Therefore, we assumed that eosinophil infiltration around the capsule of the mammary gland in the prolactin-treated mice might be attributable to the migration of larvae into the mammary gland following stimulation of the tissue-arrested larvae.

The mechanism of this stimulation of tissue-arrested larvae during breast-feeding has yet to be elucidated. In hookworm infection, tissue-arrested larvae of *Ancylostoma caninum* were activated *in vitro* by TGF-β [33]. No such connection, however, has been demonstrated in *Toxocara* infection. The secretion of TGF-β is tightly regulated by the hormones estrogen and prolactin, and they are critical factors in the tissue-specific regulation of the local production of TGF-β in the mammary gland of the rat [34]. Therefore, we presumed that a similar cytokine reaction could be induced by prolactin, and may contribute to the reactivation of cryptic larvae in *Toxocara*-infected mice.

In the present study, we found clear evidence that prolactin is one of the factors in the lactational transmission of *T. canis* larvae from mother mice to offspring. Further investigation is needed to elucidate the precise mechanism of the stimulation of tissue-arrested larvae in mice.

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RESEARCH NOTES

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An Improved Method for Recovery of Muscle-Stage Larvae from Mice Infected with *Toxocara canis*

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ABSTRACT: We report a modified digestion method that improves the recovery of *Toxocara canis* larvae from skeletal muscle. Minced muscle tissue from infected mice was incubated in artificial gastric juice for 48 hr at 37 C, and ethanol was added for the second 24 hr. This procedure allowed the larvae to be identified and counted more quickly than with the standard digestion method. This method allows measurement of the total number of larvae present in muscle tissue following oral intubation of embryonated eggs, although it does not permit counting of live larvae.

Following oral intubation of embryonated eggs, infectious-stage *Toxocara canis* larvae migrate into skeletal muscle tissue via systemic circulation. Muscle-stage larvae tend to increase in number after infection. Almost half of all recovered larvae enter skeletal muscles beyond the 10th day of infection (Oshima, 1961; Havasiova-Reiterova et al., 1995). These larvae are able to survive for long periods in muscle tissue. If an anthelmintic drug is effective against migrating larvae, the number of larvae appearing in skeletal muscle will be reduced. Therefore, for an anthelmintic trial, the number of muscle-stage larvae is a good indicator of efficacy (Fok and Kassai, 1998; Hrckova and Velebny, 2001; Horiuchi et al., 2005; Satou et al., 2005).

Both the Baermann technique and the digestion method using artificial gastric juice are used to detect larvae in skeletal muscle. The Baermann procedure, usually combined with a short-duration digestion method (less than 4 hr), permits the recovery of live larvae, but the extent of recovery is not satisfactory for estimating the total parasite burden. Additionally, since less than half of the skeletal muscle is usually employed for the digestion (Abdel-Hameed, 1984), the precise number of larvae recruited cannot be determined. In contrast, the digestion method alone permits a fairly good recovery, although a large amount of sediment remains after digestion, making the counting of larvae using stereoscopic microscopy quite time consuming. In the present report, we describe an improved method for recovering and counting larvae derived from skeletal muscle. The method is based on extended incubation in digestive fluid, followed by addition of alcohol.

Female BALB/c mice weighing 28–30 g were infected with 300 embryonated eggs of *T. canis* according to the method of Oshima (1961). Six mice were used for this experiment. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. Three weeks later, skeletal muscle tissue from each mouse was minced with 150 ml of artificial gastric juice (0.5% of 1:10,000 pepsin and 0.7% hydrochloric acid, pH 1.5). After mixing well with a blender, the minced tissue was divided into 3 equal parts. The first portion was incubated in digestive fluid for 4 hr at 37 C with vigorous agitation. The mixture was then sieved with a wire mesh (mesh diameter: 1.0 mm), and the fluid was centrifuged at 320 *g* for 5 min. The total digestion time was 4 hr (method 1). Larval counting was performed on the resulting sediment using stereoscopic microscopy. Since undigested tissues remained on the mesh after sieving, these materials were re-incubated with digestive fluid for an additional 44 hr. They were vigorously agitated and prepared for counting in the same manner as before. The second portion of minced tissue was incubated in digestive fluid for 24 hr with vigorous agitation. The solution was centrifuged as before, and the sediment was re-incubated in 50 ml of fresh digestive fluid for an additional 24 hr. No filtration with wire mesh was performed. Thus, the total digestion time was 48 hr (method 2). Larval counts in the whole sediment were performed as before. The third portion was prepared in the same manner as the second portion, but 10 ml of 50% ethanol in distilled water was added to the sediment after the second 24 hr incubation step (method 3). The number of larvae in the sediment was then counted.

Table I shows the number of larvae recovered with each procedure. There was a significant difference in larval recovery between the 4-hr digestion group and the 48-hr digestion group ($p < 0.01$). Although ethanol treatment did not significantly affect recovery, we were able to find the larvae more easily in the ethanol-treated samples. The use of alcohol in the final step has the advantages that lipid droplets, which are insoluble in trypsin-based digestive fluid, are soluble in alcohol, and that alcohol acts as a surface-tension depressant that facilitates the identification of larvae. This is reflected in the time required to complete counting of a single sample: with ethanol treatment, counting took 16.7 ± 2.5 min (mean ± SD); without ethanol treatment, counting took 33.8 ± 7.5 min. For comparison, with the sample digested for 4 hr without ethanol, counting took 91.2 ± 14.1 min. From the undigested material, we were able to find larvae after additional incubation for 20 hr and 24 hr using freshly prepared digestive fluid, suggesting that a 4-hr incubation was insufficient for the digestion of skeletal muscle.

We further assessed whether this recovery technique can be carried out by an inexperienced person (T.N.). Six BALB/c female mice were orally administered albendazole (100 mg/kg/day) suspended in olive oil for 5 days, beginning 1 day before inoculation. Six control animals were given only olive oil. Three weeks after intubation, the mice were killed, and their skeletal muscle tissue was digested using method 3, under the guidance of an experienced researcher (Z.J.). Larvae migrating to the brain were counted by squash preparation (Abdel-Hameed, 1984). At the beginning of the experiment, it took almost 3 hr to complete the counting from just 1 skeletal muscle sample, but this soon fell to 30 min. The average recovery from skeletal muscle was 56.8 ± 4.8% in

TABLE I. Number of larvae recovered from skeletal muscle tissue of mice infected with 300 *T. canis* eggs.

Digestion period (hr)			
4		48	
		Ethanol treatment	
Sediment	Undigested material	No	Yes
9.5 ± 3.0	1 ± 0.9		
10.5 ± 3.7		23.2 ± 8.3*	26.3 ± 8.5*

Six mice were used for the experiment. Numbers are given as mean ± SD. Asterisk indicates a statistically significant increase in 48-hr incubation group versus 4-hr incubation group (Student's *t*-test, $P < 0.05$).

TABLE II. Number of larvae recovered from mice inoculated with 300 *T. canis* eggs.

Albendazole*		Control†	
Skeletal muscle	Brain	Skeletal muscle	Brain
50.7 ± 22.3	38.8 ± 12.9	104.5 ± 3.5	66 ± 11.8

Larval recovery from skeletal muscle was performed using method 3.
* Six BALB/c mice were treated with 100 mg/kg/day of albendazole suspended in olive oil for 5 consecutive days beginning 1 day before inoculation.
† Six control mice were given only olive oil.

the control group versus 29.8 ± 9.8% in the albendazole group. In skeletal muscle, 104.5 ± 3.5 larvae were found in the control group versus 50.7 ± 22.3 in the albendazole group, indicating that prophylactic treatment can reduce the larvae in skeletal muscle (Table II).

The improved method described here requires substantially less operator time (since it is more than 5-fold faster) to count larvae, and the recovery is 3-fold higher than that of our previously reported methods (Horiuchi et al., 2005; Satou et al., 2005). However, the larvae recovered are no longer alive, which is likely due to the much longer incubation time required. Therefore, while this method would be suitable for measuring the efficacy of treatments that act before larval migration, it would not allow measurement of the active larval tissue burden.

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Case report

A familial case of visceral toxocariasis due to consumption of raw bovine liver

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ABSTRACT

We present 3 adult cases of visceral toxocariasis from the same family, who each consumed thin slices of raw bovine liver weekly, and developed eosinophilia and multiple small lesions in their livers and lungs. Serological examinations using the larval excretory-secretory product of *Toxocara canis* strongly indicated infection with *Toxocara* species larvae. The patients responded well to treatment with albendazole. Ingestion of raw liver from paratenic animals is considered to be a common transmission route of human toxocariasis, especially in adults.

1. Introduction

Human toxocariasis is a common helminthozoonosis caused by infestation with larvae of the nematode worms *Toxocara* (*T.*) *canis* or *T. cati* [1–5]. It has long been considered a parasitic disease that affects pet owners and children, because transmission was thought to only occur via ingestion of infective embryonated eggs after exposure to soil and hair contaminated with the feces of dogs and cats. However, infective stage larvae can also be transferred to other animals and humans through predation, and this type of parasite transfer is now considered to be frequently related to adult cases of toxocariasis in Japan [6]. Therefore, toxocariasis should be recognized as a food-borne parasitic disease, especially in societies where consumption of raw meat is prevalent. Herein, we present 3 adult cases of visceral toxocariasis from the same family who regularly consumed thin slices of bovine liver. Our findings show that consumption of raw liver from paratenic animals is an important source of infestation.

2. Cases

A 58-year-old man (Patient 1) had never been found with leukocytosis in annual medical check-up examinations until December, 2007, when an increased number of white blood cells (11,800/μl) with marked eosinophilia, absolute count 4250/μl, and elevated IgE (2345 U/ml, normal <100) were found. He was referred to Nara Medical University Hospital. At the initial interview, the patient noted that he and 2 other family members, his 57-year-old wife (Patient 2) and 27-year-old son (Patient 3), consumed raw bovine liver every Friday for the past year, believing that it was good for their health. Their habit was to obtain 100 g of raw bovine liver at a nearby meat shop and serve it as thin slices at dinner. Patient 1 generally consumed the most, followed in order by Patient 2 and Patient 3. In contrast, the mother of Patient 1, who lived in the same house, only ate the raw liver on a few occasions.

We performed blood examinations for all 4 family members. Although none was symptomatic, the 3 regular consumers showed increased eosinophils and IgE (Table 1), while the mother who consumed raw liver only rarely showed no eosinophilia or elevated IgE. Results of a blood examination for Patient 2 obtained by a local physician 1 year previously, prior to beginning the dietary habit, showed a normal number of white blood cells at 6000/μl with a 3%

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Table 1
Patient laboratory data

	Patient 1	Patient 2	Patient 3
WBC (/μl)	11,800	8700	8800
Eo (%)	36.0	27.0	19.3
Hb (g/dl)	14.7	14.5	15.6
PLT (× 10^4/μl)	28.4	25.4	29.0
CRP (mg/dl)	0.2	0.5	0.1
AST (IU/l)	23	24	17
ALT (IU/l)	12	28	22
ALP (IU/l)	279	227	201
γGTP (IU/l)	35	53	21
IgE (U/ml)	2345	645	422

Abbreviations and normal ranges:
WBC: white blood cells, 3900–9800/μl.
Eo: eosinophils, 0–5%.
Hb: hemoglobin, 13.2–15.6 g/dl.
PLT: platelet, 13.0–36.0 (× 10^4)/μl.
CRP: C-reactive protein, less than 0.2 mg/dl.
AST: aspartate aminotransferase, 12–32 IU/l.
ALT: alanine aminotransferase, 5–36 IU/l.
ALP: alkaline phosphatase, 120–360 IU/l.
γGTP: gamma-glutamyl transpeptidase, 11–69 IU/l.
IgE: immunoglobulin E, less than 100 U/ml.

eosinophil fraction, though IgE was not examined. Additional tests were performed to determine the etiology of the hypereosinophilia in the patients. Chest computed tomography (CT) demonstrated multiple small pulmonary lesions, nodules with halos and poorly defined margins, and ground-glass opacity with a poorly defined margin in all. Furthermore, contrasted abdominal CT in the portal phase revealed multiple, poorly defined, low-attenuated nodules in the liver of Patient 1, while Patients 2 and 3 each had only a single lesion. Representative CT images from Patients 1, 2, and 3 were shown in Fig. 1. Some nodules in the liver of Patient 1 showed peripheral rim enhancement in the arterial phase (Fig. 2A), while most nodules were undetectable in the equilibrium phase. These CT findings of pulmonary and hepatic lesions were very consistent with those in previous reports of toxocariasis [7–9]. Ultrasonography (US) detected multiple, small, oval, hypoechoic lesions in the liver of Patient 1, and 3 hypoechoic lesions in the liver of Patient 2 including the one lesion detected by CT, whereas none was detected in Patient 3. We also performed contrast US using a newly developed material, Sonazoid® [10], and compared those images with the CT images (Fig. 2B). The lesions were detected as hypoechoic areas in the portal phase and even more clearly in the equilibrium phase, while they were not enhanced in the arterial phase, suggesting that the lesions were poorly supplied with arterial or portal blood. In the post-vascular or so-called Kupffer image phase, the lesions remained un-enhanced, suggesting the absence or scant presence of Kupffer cells (Fig. 2C).

A rapid diagnostic test for toxocariasis, ToxocaraCHECK® [11], which detects IgG antibodies against the larval excretory–secretory (LES) product of *T. canis* on an antigen-sensitized nitrocellulose membrane, showed positive results for all 3 patients. Furthermore, a microplate enzyme-linked immunoabsorbent assay (ELISA) using the LES product and serum from each patient diluted 1:900 revealed the presence of human IgG antibodies at very high titers. The optical density (OD) values at 405 nm for sera from Patients 1, 2, and 3 were 1.58, 1.41, and 1.38, respectively, as compared to the established OD value cutoff level of ≤0.2 for serum from healthy individuals. We also examined immunopositivity against nematode antigens other than the LES product of *T. canis* using a gel diffusion test (Fig. 3), which revealed a strong positivity against the LES products of both *T. canis* and *T. cati*, suggesting a high cross-immunogenicity between them or dual infection, though no formation of precipitate was observed against the LES product of *Ascaris suum* or *Anisakis simplex*. Since no serological examination has been established yet to discriminate between toxocariasis caused by *T. canis* and that by *T. cati*, we made a diagnosis of toxocariasis by *Toxocara species* for all 3 patients.

The patients were instructed regarding prevention of re-infection and treated with a 4-week regimen of daily albendazole at 600 mg (10.8 mg/kg of body weight for Patient 1, 12.8 mg/kg for Patient 2, 10.0 mg/kg for Patient 3). All completed the treatment, though a mild elevation of transaminases up to double the upper limit was observed in Patient 2. During treatment, the eosinophil count decreased in each and became normalized by the end of treatment in Patient 2, while Patients 1 and 3 were further treated with albendazole at the same dose for two more weeks until the eosinophil count became normalized. Hepatic and pulmonary lesions were undetectable by CT and US examinations at the end of treatment in all of the patients. Three months after finishing the treatment with albendazole, we confirmed that a normal eosinophil count was maintained in each patient, along with no recurrent hepatic or pulmonary lesions in CT findings. In addition, the OD values of anti-*T. canis* LES were decreased to 0.95, 0.80, and 0.74 from the initial values of 1.58, 1.41, and 1.38 before treatment in Patients 1, 2, and 3, respectively.

3. Discussion

Visceral toxocariasis is a representative infection of visceral larva migrans (VLM), first reported by Beaver et al. [12], known to be prevalent among preschool children, as they tend to play with dogs in open areas and ingest egg-contaminated soil. However, a recent review of human toxocariasis cases in Japan noted that the disease affects predominantly adults rather than children [6].

There are a number of case reports of adult toxocariasis [13–21], and accumulating evidence [22–27] has revealed that a common route of adult human infection is through ingestion of uncooked or raw liver from a paratenic host. In general, transfer of infective stage larvae through predation is a common mode of helminth transmission among carnivorous vertebrates and this type of parasite transfer can also occur from animals to humans. In experiments with chicken, cattle, and swine, Taira et al. found that the animals were able to function as paratenic hosts for *T. canis* and that the liver was one of the most intensely affected organs [22,23]. Similar observations regarding the importance of predatory cycle have also been reported for cases of infection with *A. suum* [28,29].

Adults with a dietary habit of consuming raw liver have been found to be at high risk for human VLM [24–26]. Morimatsu recently reported an interesting familial case in Japan, in which a father (71 years old) and son (45 years old) developed visceral toxocariasis after consumption of raw chicken livers, and found *T. canis larvae* in the livers of chickens raised in their breeding farm [17]. The present patients began to eat raw bovine liver weekly and continued the habit for about 1 year. Patients 1 and 2 had normal white blood cell counts including eosinophils in routine peripheral blood examinations conducted 1 year and just prior, respectively, to beginning the weekly consumption of raw bovine liver, which suggests that the dietary habit of eating raw liver contributed to toxocariasis in those cases. We strongly suspect that some of the raw liver served at dinner was infected with larvae of *Toxocara species*. Thus, it is important to recognize that toxocariasis can be a food-borne parasitic disease, based on the present findings.

The majority of patients with visceral toxocariasis are asymptomatic and the disease is often discovered during investigation of peripheral eosinophilia [8,9,30], as in the present cases, though those with a high number of worms may complain of vague abdominal discomfort, abdominal pain, cough, dyspnea, fever, or general weakness. Although each of our patients were asymptomatic, the degree of eosinophilia, serum IgE level, and number of hepatic lesions were prominently high in Patient 1, who ingested larger quantities of raw liver as compared to the others, suggesting that the number of worms and disease severity may be proportional to the amount of raw liver intake.

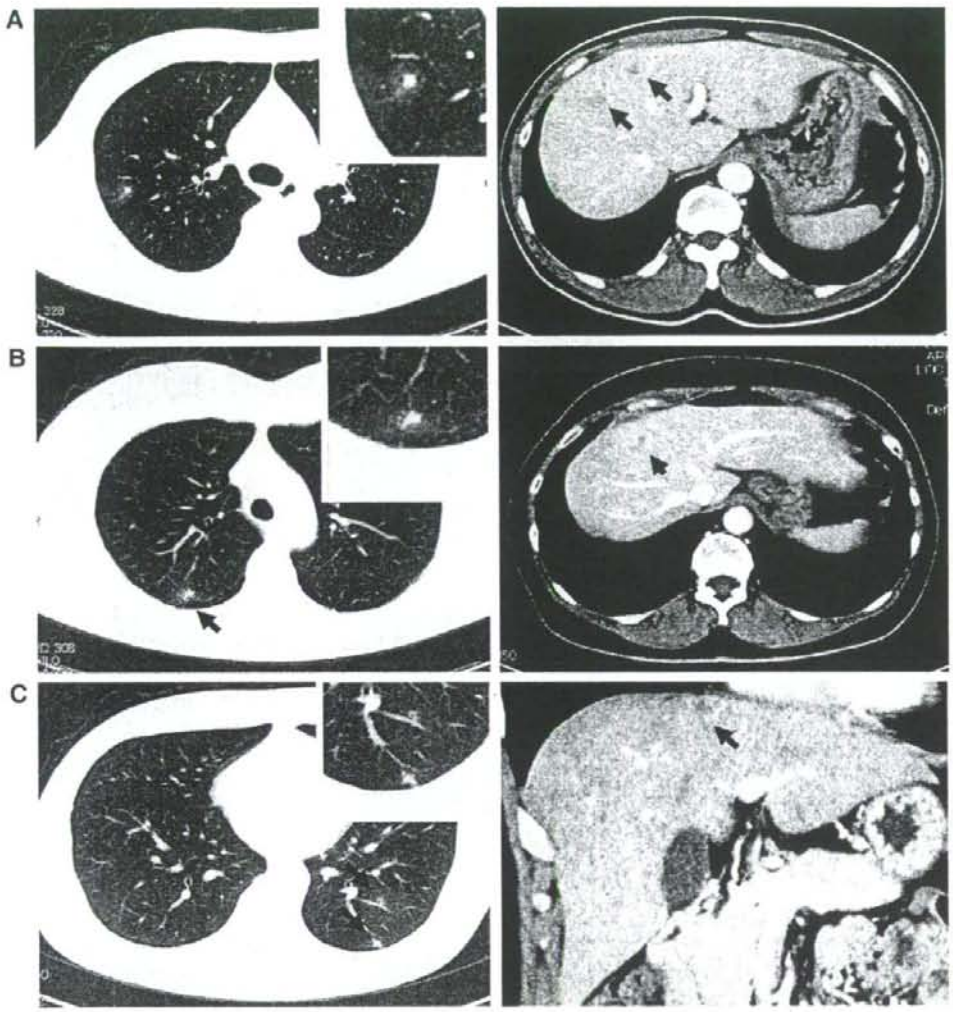

Fig. 1. CT images of pulmonary and hepatic lesions. A, B, and C show representative CT images from Patients 1, 2, and 3, respectively. Pulmonary lesions (arrows, magnified in inset) were shown as nodules with halos and a poorly defined margin or ground-glass opacity with a poorly defined margin. Hepatic lesions (arrows) appeared as small, poorly defined areas of low-attenuation in the portal venous phase of contrast-enhanced CT scanning.

We found that imaging modalities were very useful to reach a diagnosis. Characteristic CT findings of hepatic and pulmonary lesions in visceral toxocariasis reported elsewhere [7–9] are compatible to those found in our patients. Typically, the hepatic lesions are multiple, small (usually less than 2 cm in diameter), poorly defined, oval or elongated, and with low attenuation, and usually best visualized in the portal venous phase of contrast CT. Pulmonary lesions are shown as multiple small nodules (mostly less than 3 cm in diameter), with some associated with halos with poorly defined margins, and also shown as ground-glass opacity with a poorly or well-defined margin. Lesions in the liver and lung tend to be found in the periphery of those organs. In the present cases, we also performed US examinations using Sonazoid, a recently developed microbubble contrast agent, which is phagocytosed by liver-specific macrophages, known as Kupffer cells, following the vascular phase [10]. Sonazoid-contrast US showed that the liver lesions were poorly supplied with arterial and portal blood, and contained no or few Kupffer cells as compared with the surrounding liver parenchyma. These CT and US image findings are compatible to inflammatory granuloma. Although we did not perform a puncture biopsy of the hepatic lesions for histological examinations, eosinophilic granuloma would be expected.

A serological examination is also important for an accurate diagnosis, as it is difficult to obtain worms from patients in most cases. In the present cases, we performed 3 kinds of serological tests, rapid screening ELISA, quantitative ELISA, and an immunodiffusion test, using the LES product of *T. canis*, which is known to be highly immunogenic. Sera from the 3 patients were positive in all of those tests. However, in immunodiffusion tests with LES products of *T. canis*

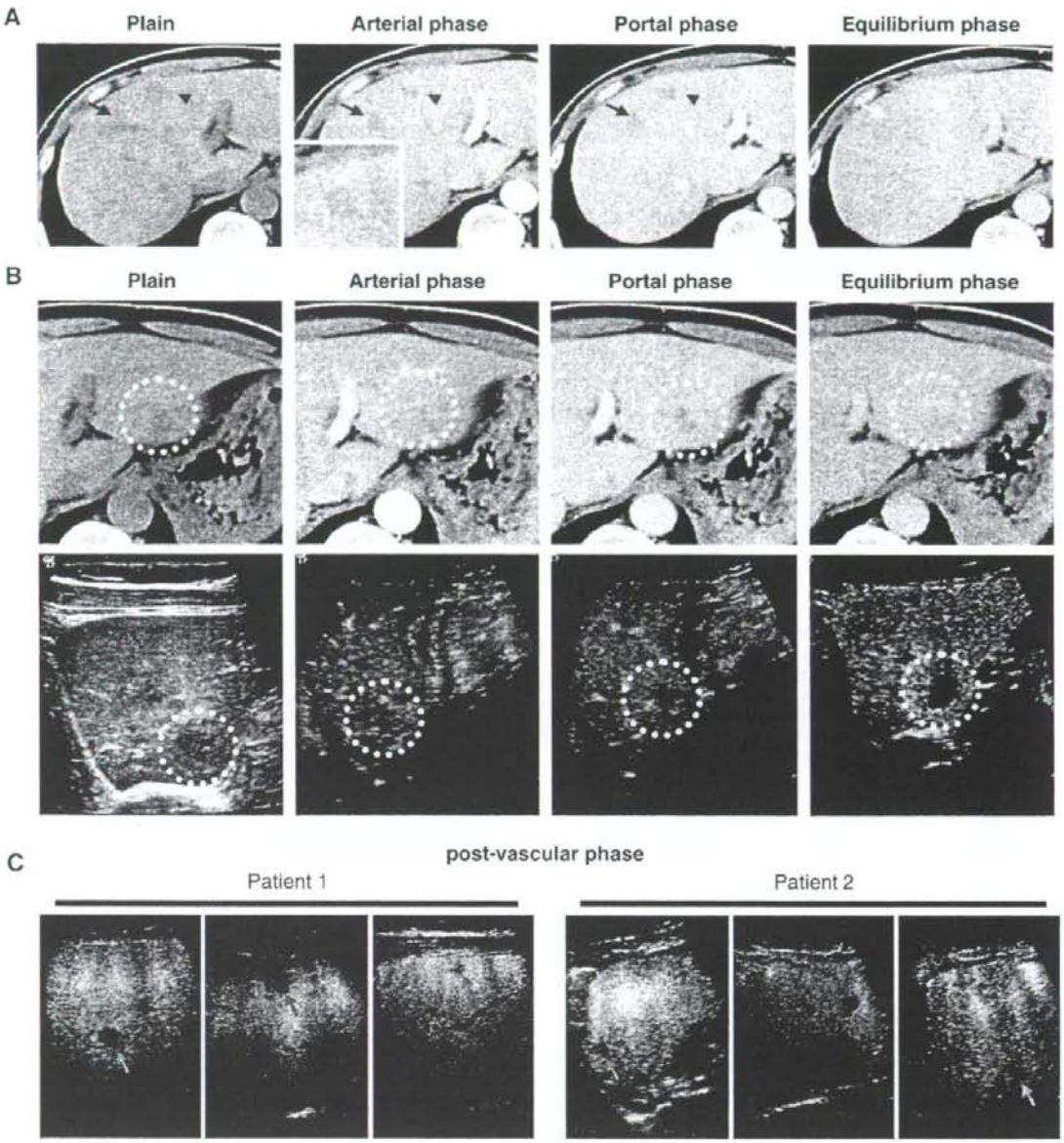

Fig. 2. CT and US images of hepatic lesions. A. Two nodules (arrow and arrowhead) in the liver of Patient 1 are shown. That shown by the arrowhead (magnified in inset) had weak peripheral rim enhancement in the arterial phase, while both were undetectable in the equilibrium phase. B. A lesion (circle with broken line) in the left lobe of Patient 1 was targeted with Sonazoid-contrast US (lower) and the results were compared with contrast CT images (upper). With CT, the lesion was best seen in the portal phase and became undetectable in the equilibrium phase, while it was clearly shown as an un-enhanced area in the equilibrium phase. C. Post-vascular Sonazoid-contrast US images revealed that the lesions (arrows) remained hypoechoic. Three lesions each from Patients 1 and 2 are shown.

and *T. cati*, sera from the patients were reactive to both of the LES products, because of their high cross-immunogeneicity. Finally, we made a diagnosis of toxocariasis by *Toxocara species*.

Covert toxocariasis with eosinophilia alone is often treated conservatively after instruction regarding prevention of re-infection. Stopping the habit of ingesting raw liver alone might have been adequate for the present cases of asymptomatic toxocariasis. However, the existence of living larvae in the lungs and liver for a prolonged period is a potential risk for their migration to other organs, including the spinal cord and brain, leading to serious complications. We decided to prescribe albendazole, which is commonly used for toxocariasis and known to be effective with minimal adverse reactions. A dose of

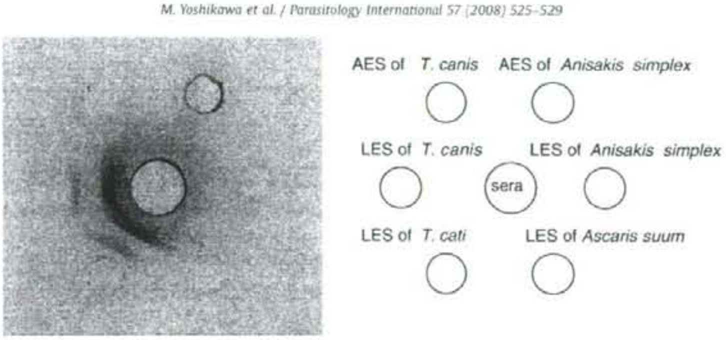

Fig. 3. Results of serological tests. Data from Patient 1 shown with schematic positions of antigens and sera are presented as representative findings. The antigens used were adult worm extract (AES) of *T. canis*, AES of *Anisakis simplex*, larval excretory-secretory (LES) of *Anisakis simplex*, LES of *Ascaris suum*, LES of *T. cati*, and LES of *T. canis*. Strong precipitin bands were observed for the LES products of *T. canis T. cati* in serum samples from all 3 patients.

400 mg of albendazole twice a day or 10 mg/kg of body weight/day in two divided doses for 5 days seems to be the currently recommended therapy [4,31,32], though the optimal duration of therapy is unknown [33]. According to a previous report [31], only 32% of patients with toxocariasis were clinically cured with a 5-day regimen and other reports have noted that additional treatments with other anthelminthic drugs, such as diethylcarbamazine and mebendazole, or the use of albendazole for a longer period was effective [18,34–39]. We adopted a 4-week regimen of daily albendazole at 600 mg, with the disappearance of eosinophilia considered to mark the endpoint of therapy. Clinical improvement appeared soon after the initiation of treatment, demonstrated by a decrease in eosinophil count, with minimum adverse effects related to mild liver dysfunction, followed by the disappearance of hepatic and pulmonary lesions.

Based on our results, we concluded that infestation with *Toxocara species* from paratenic animals is likely a common and important mode of transmission to humans, especially adults, in areas such as eastern Asia where the consumption of raw liver remains a cultural habit.

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INTERNAL MEDICINE

□ PICTURES IN CLINICAL MEDICINE □

Visceral Toxocariasis from Regular Consumption of Raw Cow Liver

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Key words: toxocariasis, visceral larva migrans (VLM), albendazole, mode of transmission, raw liver

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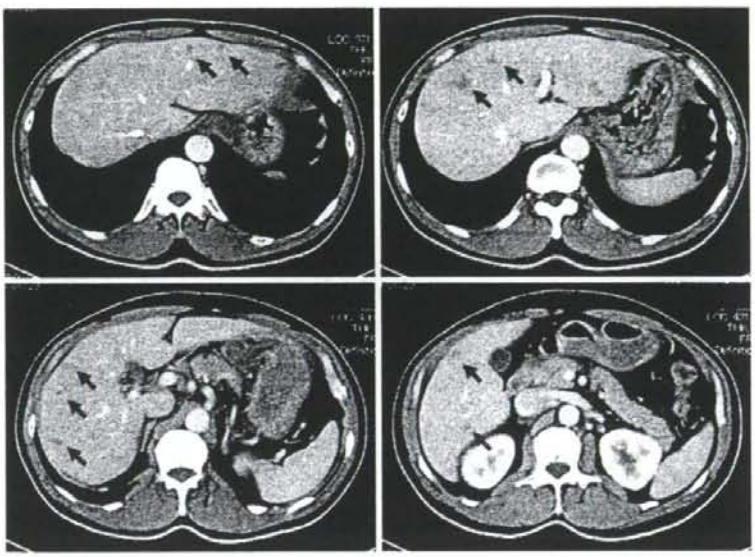

Picture 1. Contrast-enhanced CT scan image obtained at portal venous phase showing multiple small, ill-defined, and low-attenuation lesions in the liver of the patient (arrows).

A 58-year-old man had leukocytosis (leukocytes 11,800/μL), with marked eosinophilia (36%) and an increased total IgE at 2,345 U/mL (normal <100). There were no abnormal results in his annual check-up examinations including blood parameters until the most recent examination, when he began to eat raw cow liver weekly. Abdominal computed tomography (CT) revealed multiple, ill-defined, low-attenuated lesions in the patient's liver (Picture 1). Chest X-ray images did not reveal apparent abnormalities, whereas chest CT demonstrated a nodule with a halo and ill-defined margin, and ground-glass opacity (Picture 2). Gel diffusion test using the patient's serum revealed strong precipitin bands

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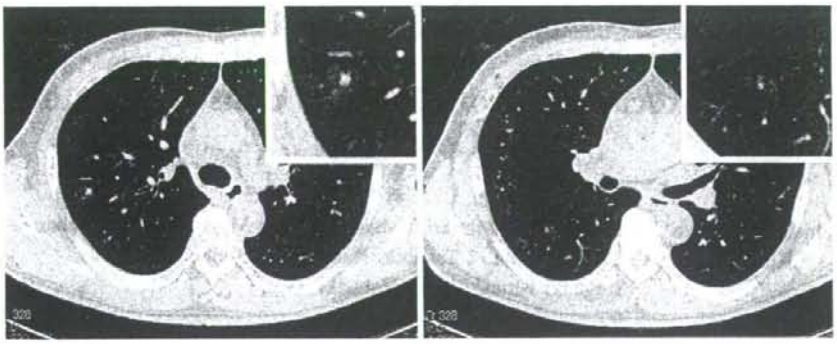

Picture 2. Chest CT scan image showing nodule with halo and ill-defined margin (left), and groundglass opacity with an ill-defined margin (right). The lesion is shown magnified in the inset.

against larval excretory-secretory (LES) products of both *Toxocara* (*T.*) *canis* and *T. cati*, thus visceral larva migrans (VLM) was highly suspected. Ttreatment with albendazole was performed. Consumption of paratenic meat, especially raw liver, was the suspected source of infestation.

Vol. 24, No. 9, 2008 化学療法の領域
特集 寄生虫感染症

6．イヌ回虫症

赤尾 信明*

イヌ回虫症は人畜共通感染症として重要な寄生虫症である。小児のみならず，近年は成人の感染例が増加している。感染源も多様化し，虫卵で汚染された砂場や感染犬の被毛に付着した虫卵以外に，ニワトリやウシなどの生獣肉，肝臓の生食による感染例が多くなってきている。イヌ回虫症は多彩な病態を呈し，ネフローゼ症候群や関節リウマチ様関節炎，血球貪食症候群等とも合併し，診断が困難な症例もみられる。幼虫を病理組織学的に検出した例は少ないが，幼虫排泄物抗原を用いた抗体検査によって診断が確定することも多い。アルベンダゾールによる治療が推奨されているが，眼型イヌ回虫症では効果のないことが多く，新たな治療薬の開発が望まれる。

Key Words：イヌ回虫／トキソカラ症／感染源／病態

Ⅰ はじめに

イヌ回虫症とは，イヌ科の動物を終宿主とする回虫の幼虫がヒトに感染したときに起こる病気で，ヒトの体内で成熟したり増殖したりすることはない。成虫は子イヌの消化管の中に寄生しており，子イヌが成長するにつれて消化管から自然に排虫されてしまう。それゆえ感染源としてはイヌ回虫に感染している子イヌが重要である。免疫能の正常な成犬にイヌ回虫が感染しても体内で成熟することなく，全身の組織内で肉芽腫を形成して，幼虫はこの中で『冬眠』状態のまま長期間生きている。そして幼虫は母イヌの妊娠を契機として肉芽腫から脱出して子宮内の胎児に侵入し，出産と同時に肺臓に移行して発育を始める。母イヌから子イヌへの感染には乳汁を介した感染も知られているが，母子イヌ間の感染は経胎盤感染が主導であると考えられている。

ヒトがイヌ回虫幼虫包蔵卵を誤って経口的摂取すると，消化管内で虫卵は孵化し，幼虫は粘膜から組織内に侵入して血流に乗り，門脈を経由して肝臓に至る。その後，肺臓から全身の臓器に移行し，さまざまな病害を及ぼす。このように寄生虫の幼虫がヒトに侵入して起きる病気を幼虫移行症といい，ヒトを終宿主としない多くの動物由来寄生虫でも同様な病態が起きる。しかしイヌ回虫症はペット由来の寄生虫感染症の中でも，もっともよく知られたもののひとつであり，国内からも多くの症例が報告されている。本稿では，イヌ回虫症の最近の話題について解説する。

Ⅱ 感染源としての子イヌ

イヌ回虫は子イヌの小腸に寄生しており，成犬になるにつれて自然に排虫されていく。2007年度に我々が行った栃木県動物愛護センターに搬入された3カ月齢の子イヌ43頭の糞犬検査では，29頭（67%）からイヌ回虫卵が検出されており，子イヌの糞便がイヌ回虫症の感染源として重要である傾向に変化はない。一方，内田ら（1995年）の調査では感染犬の被毛には虫卵が高率に付着し

Toxocariasis

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ており，これが感染源になる可能性に言及している[1]。アイルランドと英国での調査でも，検査した60頭のイヌのうち15頭の被毛から71個の虫卵が検出され，その内の3頭は幼虫包蔵卵にまで発育していたという[2]。また獣医師や動物看護師を対象とした血清疫学調査でも，これらの職業のヒトたちはイヌ回虫に対する抗体保有率が有意に高いことが報告されている[3][4]。

Ⅲ イヌ回虫症の臨床像

イヌ回虫症はその寄生部位から，内臓型，眼型，神経型，潜在型の4型に分類されてきた[5]。内臓型イヌ回虫症は，肝腫大と発熱，好酸球増多を特徴とし，眼型は，ぶどう膜炎や眼底の腫瘤性病変をともなうが，末梢血中の好酸球増多をともなうことは少ない。また肝腫大や発熱も通常みられず，飛蚊症や視力低下，視野異常を自覚して発見される例が多い。まれに全眼球炎を起こし失明に至ることもある。またイヌ回虫幼虫は中枢神経系に移行して長期間寄生することができることと，ヒトの疫学調査でイヌ回虫特異抗体の陽性率と，てんかんあるいは神経症の既往を持つグループとの間に正の相関関係がみられるという報告があることから，神経型トキソカラ症が区別されている。潜在型トキソカラ症は内臓型トキソカラ症と較べて肝腫大や好酸球増多は顕著ではないが，腹痛や喘息，食欲不振といった不定愁訴を訴える患者の抗体検査で，特異抗体が正常人よりも有意に上昇しているものをいう。潜在型は感染幼虫数が少なく慢性に経過した場合にみられ，アレルギー疾患を併発しやすいといわれている[5]。このような典型例以外にも，ネフローゼ症候群や関節リウマチ様関節炎，血球貪食症候群と合併した非典型例も報告されている[6]~[8]。

このような従来からの分類をふまえ，ここでは最近のイヌ回虫症の臨床所見に注目して解説を試みる。

Ⅳ 診断

確定診断には組織内から幼虫を直接検出するか，その断端を病理組織学的に証明することが必要である。しかし国内でこれまで，幼虫が確実に検出された症例は**表1**に示すように3例で，虫体の断片様の物体がみつかりこれを幼虫と判断した疑診例は2例ある。

一方，臨床所見や血液検査にくわえて，血清や眼内液中の抗体検査結果に基づいて診断され医学中央雑誌に収載されたイヌ回虫症例は，**表2**に示すように過去12年間だけで129例にのぼっている。この中には，血清学的にブタ回虫幼虫による感染が疑われた症例が15例含まれている。

抗体検査には抗原特異性の高いイヌ回虫幼虫排泄物（*Toxocara canis* larval excretory-secretory antigen：TES）を用いて，ELISA法や寒天ゲル内二重拡散法，あるいは迅速診断キットなどが行われているが，悪性腫瘍の患者の中にはこの抗原と交叉反応を示すものもあり注意が必要である[9]。

Ⅴ 食品媒介感染症としてのイヌ回虫症

1980年8月に発熱と体重減少を主訴とした57歳の男性が福岡県内の病院を受診した。患者は6月末に同僚3名とともに自宅で飼育していた鶏の肝臓を生食し，直後から，下痢，腹痛，嘔吐が出現したが，これらの食中毒症状は数日で軽快したため放置していたところ，主訴が出現したので来院したという。血液検査の結果，末梢血液中の好酸球数が58%を上昇しており，総免疫グロブリン（Ig）E量も正常の10倍以上に増加していた。TES抗原に対する血清中の抗体が強陽性反応を示したことから，イヌ回虫幼虫による内臓型幼虫移行症と診断された[10]。

ニワトリあるいはウシの肝臓を生食後に同じような経過を辿った2症例が，同じグループによって報告された。いずれも男性で，全身倦怠を主訴

TES（*Toxocara canis* larval excretory-secretory antigen：イヌ回虫幼虫排泄物）
Ig（免疫グロブリン）

表1　イヌ回虫幼虫あるいは幼虫様組織が確認された症例

	患者	雑誌名	臨床診断	病変部位	幼虫確認	報告者
確診例	8歳女児	臨床眼科	左眼の網膜膠腫	網膜肉芽腫	摘出眼球　幼虫断端	吉岡（1966年）
	成人女性	Lancet	好酸球性肺炎	皮疹	皮膚生検　幼虫断端	Aragane, et al（1999年）
	成人女性	私信		頸髄	頸髄生検　幼虫断端	大津市民病院（2008年）
疑診例	成人女性	臨床眼科	ぶどう膜炎	硝子体*	硝子体手術　摘出標本	伊集院ら（1999年）
	成人女性	臨床寄生虫誌	ぶどう膜炎	硝子体*	硝子体手術　摘出標本	赤尾ら（2004年）

*硝子体液中のイヌ回虫幼虫排泄物抗原に対する抗体陽性

表2　過去12年間のイヌ回虫症症例数と抗体検査依頼数

発表年	症例数*	内ブタ回虫幼虫が原因とされた症例	抗体検査依頼数**
2008	4	0	18
2007	5	0	24
2006	17	1	32
2005	11	0	39
2004	13	0	25
2003	25	3	35
2002	9	1	52
2001	6	2	70
2000	10	3	56
1999	10	0	61
1998	9	2	81
1997	10	3	36
合計	129	15	529

*医学中央雑誌収録の論文に記載された症例数
**東京医科歯科大学に抗体検査依頼のあった症例数

図1　イヌ回虫感染3日目のマウス肺臓
両肺野に多数の出血斑を認める。

に来院し，検査の結果，好酸球増多と血清中のTESに対する抗体が上昇していた。その後もニワトリ，牛，シャモの肝臓の生食後に，全身倦怠，発熱，咳嗽など多彩な症状を呈した症例が相次いで報告されている。これらの症例はイヌ回虫症が獣肉や肝臓の生食によって感染する「食品媒介寄生虫症」であることを明確に示している。1999年に食品衛生法が一部改正され，食中毒原因物質として「クリプトスポリジウム，サイクロスポーラ，アニサキス等」の寄生虫種が追加されたことにより，獣肉などが感染源であると特定されたイヌ回虫症については食品衛生法上の食中毒事案として保健所に届け出る必要があると思われる。

Ⅵ　肺炎症状を初発とするイヌ回虫症

マウスを用いた実験では，イヌ回虫幼虫は肝臓から血流に乗って肺臓に至り出血性の肺炎を起こす（図1）。このような重度な出血性肺炎も一過性に経過し，3週間後には完全に消失する。

ヒトのイヌ回虫症でも同じような肺炎症状が急

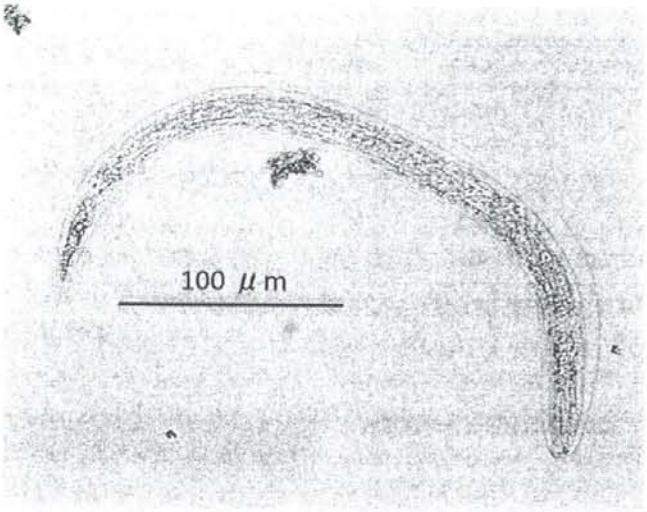

図2　イヌ回虫幼虫
自宅の庭で飼育していたニワトリの肝臓を生食して発症した親子例(75歳と45歳)で，患者が生食した残りの凍結保存されていたニワトリの肝臓から回収されたイヌ回虫幼虫。

起される。Morimatsuら（2006年）が報告した症例は，自宅で飼育していたニワトリの生肝臓を75歳と45歳の親子で喫食し，3週間後に，発熱，倦怠感，頭痛，呼吸困難を訴えた。両名とも肺野には小結節陰影が多発し，時間経過とともにこれらの肺炎病巣は移動していった[11]。気管支洗浄液(BAL)中には多数の好酸球がみられ，血清のみならず気管支洗浄液中にもTES抗原に対する抗体が証明された。イヌ回虫幼虫による好酸球性肺炎が疑われる症例では，BAL中の抗体検査が診断に有用であることが示唆された症例である。またこの親子例では，冷凍保存されていたニワトリの肝臓からイヌ回虫幼虫が多数検出されている（**図2**）。

発熱，咳嗽を主訴とし，好酸球増多と総IgEの上昇がみられ，獣肉の生食歴がある患者については，胸部CT検査の結果，境界不鮮明で，ハローをともなった小結節が短期間で消退をくり返す場合は，イヌ回虫症も念頭に抗体検査を実施すべきである[12]。

Ⅶ　皮疹を起こすイヌ回虫症

ヒトに幼虫移行症を起こす顎口虫や旋尾線虫幼虫は，幼虫が皮下に出現して爬行疹を起こす。このように，ある種の寄生虫症で虫体が直接の原因となって皮膚病変を起こすことが知られている。一方，アニサキス抗原に感作されたヒトでは，次にアニサキス幼虫が感染すると全身の蕁麻疹様皮疹をともなうことがある。イヌ回虫症でもしばしば皮疹のみられることが報告されているが，その機序は幼虫が皮膚に移行して起きる直接的な結果ではなく，感染にともなう免疫応答によるものと考えられてきた[13][14]。しかしAragane(1991年)は，ウシ肝臓の生食歴のある患者で皮膚に生じた痒疹の生検標本中に，好酸球性膿瘍の中心にイヌ回虫幼虫を発見し，この幼虫が皮下織に移行して皮膚炎が生じることを報告した[15]。イヌ回虫幼虫による慢性蕁麻疹の発症機序についてはまだ不明な点が多く残されており，今後さらに詳しい検討

BAL（気管支洗浄液）

が必要である。

Ⅷ　小児イヌ回虫症と成人イヌ回虫症

イヌ回虫症は，感染源となる子イヌに触れる機会が多く，かつ手洗いの不十分なことが多い小児の感染症であると考えられてきた。また多くの砂場の砂からイヌ回虫あるいはネコ回虫卵が検出されることからも，戸外で遊ぶ機会の多い子どもの病気であるとみなされてきた。Barriga (1988年) は，内臓型イヌ回虫症の好発平均年齢は9.5歳で，成人での発症は18%であったとしている[16]。眼型についても，Wilder (1950年) が46例の小児の摘出眼球のうち26眼からイヌ回虫の幼虫断端をみつけて以来，半世紀以上に渡って眼型イヌ回虫症も小児に多い疾患であると思われていた。しかしYoshidaら (1999年) は，38例の眼型イヌ回虫症のうち34例 (89%) が20歳以上の成人であったと報告している[17]。過去12年間に，我々の研究室にイヌ回虫症が疑われて抗体検査の依頼があった529例のうち，性別と年齢の記載のあった444例について集計してみると，12歳以下の児童は47例と10.6%を占めるに過ぎなかった。眼型イヌ回虫症の134例だけをみても，12歳以下の患者は男女ともに7例で，全症例に占める割合は10.4%であった。このように最近のイヌ回虫症は，かつて言われていたような小児の感染症ではなく，獣肉の喫食や虫卵で汚染された土壌に触れる機会のあるどんな年齢層にも発症する可能性のある疾患であると考えなければならない。

Ⅸ　髄膜炎・脊髄炎型イヌ回虫症

イヌ回虫幼虫は中枢神経系にも移行するが，ヒトを対象とした症例対照研究では，イヌ回虫抗体陽性者で神経症状を呈する例はそれほど多くはない。しかし中には，髄膜炎や脊髄炎などの重篤な症状を起こす例も知られている。太田ら (1994年) は，前頭部痛と発熱，けいれんを主訴に受診した21歳の女性例を報告している。患者は長年にわたってイヌと密接な接触歴があり，検査の結果，髄液中にTESに対する抗体が証明された[18]。また吉良ら (2006年) は，四肢のしびれや感覚低下を主訴とし，脊髄MRIで病巣が描出される6症例を報告し，寄生虫性脊髄炎あるいはアトピー性脊髄炎という新しい疾患概念を提唱している[19]。脊髄炎型イヌ回虫症は九州地方に多くみられ，ブタ回虫成虫抗原を使った抗体検査でも陽性反応を示すことから，ブタ回虫幼虫移行症として報告されている例も多い。最近，しびれなどの神経症状を呈し，滋賀県下の病院で手術された患者の頸髄からイヌ回虫幼虫断端が病理組織学的に確認され (私信)，被圧迫性脊髄症の鑑別診断として脊髄炎型イヌ回虫症は重要な疾患であると考えられる。

Ⅹ　イヌ回虫以外の動物由来回虫による感染症

イヌ回虫以外にもヒトへの感染が報告されている動物由来回虫には**表3**に示すものがある。またヒトへの感染は確認されていないが，感染する可能性があるものには，クマ回虫，タヌキ回虫がある。

このうちアライグマ回虫の感染は致死的な経過を辿る。また網膜内に侵入すると瀰慢性片眼性亜急性神経網脈炎を起こし，失明に至る。国内の野生アライグマでの感染はいまだ報告はないが，動物園で飼育されているアライグマに感染がみつかっており，今後とも監視を強化していく必要がある。アライグマ回虫幼虫はヒトを含む非固有宿主内で発育し，感染後4週目では3～4倍に成長する。このように大きく成長した幼虫による機械的な中枢神経系の破壊と，排泄物に対する炎症反応が病変をより重篤なものにしている可能性が考えられている (**表3**)。

ネコ回虫の成虫はイヌ回虫とはその頭部の頸翼の大きさから簡単に区別することができる。そのためヒトがネコ回虫成虫を吐出あるいは排泄したという報告はいくつかある。国内でも5歳男児が3隻の虫体を吐出した例が報告されている。そのためネコ回虫はヒトの体内である程度発育が可能ではないかと推測する報告もある。しかしこれまでヒトがイヌ回虫成虫を吐出したという報告はいくつかあるが，いずれも間違った観察に基づくも

表3 イヌ回虫以外の動物由来回虫類とその病変

		ヒトでの病変
ヒト感染例あり	ネコ回虫	イヌ回虫症に類似。まれに成虫が寄生
	ブタ回虫	イヌ回虫症に類似
	アライグマ回虫	致死的脳炎，稀慢性片眼性亜急性神経網膜炎
	コウモリ回虫	肝炎類似疾患
	小兎唇回虫	頭頸部の皮下膿瘍
		動物モデルでの病変
ヒト感染例なし	クマ回虫	アライグマ回虫症に類似
	タヌキ回虫	肝炎類似疾患，幼虫は肝臓に限局

のであろうとされている。

一方，ヒトに感染する時期の幼虫を形態学的に区別することはそれほど容易ではない。ましてや免疫学的にイヌ回虫症とネコ回虫症を鑑別することは両者の幼虫由来TESの抗原性がきわめて類似しているので困難である。そのため，これまで成虫抗原を用いてネコ回虫による感染であると報告されているいくつかの症例については，今後見直す必要があるかもしれない。

XI イヌ回虫症の治療

単に血清抗体が陽性でほかに症状がない場合には治療の必要はない。好酸球数の異常高値や活動性の感染が疑われる場合は治療の対象となる。治療には，アルベンダゾール10～15mg/kg/日，分2～3を4～8週間経口投与する。しかし肝機能障害が高い頻度で出現するので，投薬期間中は注意深い観察が必要である。内臓型のみならず脊髄型イヌ回虫症でも良好な反応が期待できる。

眼型イヌ回虫症におけるアルベンダゾールの治療成績については一定していない。眼トキソカラ症の新しい動物モデルであるスナネズミを用いた我々の検討でも，眼内に出現した幼虫に対してアルベンダゾールは何ら効果を示さなかった。しかしステロイドの眼注は炎症を抑制した（未発表）。そのためヒトでは駆虫薬はステロイドとともに投薬する。また病変の拡大を阻止するために光凝固や冷凍凝固術を考慮する。ぶどう膜炎が遷延した場合には硝子体手術が必要である。この際，硝子体液を採取し抗体の有無を検査することは，眼型イヌ回虫症の診断に重要である。中枢神経系や眼内に移行した幼虫に対する新たな駆虫薬の開発が望まれる。

XII おわりに

イヌ回虫症は先進国や開発途上国を問わず，世界中に広く分布する感染症であり，我々の身近に暮らすペットから感染する寄生虫症としても重要である。ヒトへの感染を未然に防ぐためには，子イヌの時期における駆虫の徹底と，砂場遊びのあとの手洗い励行，さらには生獣肉の喫食習慣を中止することによって感染を予防することができる。

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