J. Parasitol., 82(5), 1996, p. 835–837

Re-Migration of Fifth-Stage Juvenile *Dirofilaria immitis* into Pulmonary Arteries after Subcutaneous Transplantation in Dogs, Cats, and Rabbits

M. Hayasaki, Department of Internal Medicine and Parasitology, School of Veterinary Medicine, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

ABSTRACT: An assessment was made of the capacity for re-migration of the 5th-stage juvenile canine heartworm, *Dirofilaria immitis*. Live, 5th-stage juvenile worms recovered from the pulmonary arteries of infected dogs were transplanted into the subcutaneous tissue of uninfected dogs, cats, and rabbits. A mean of 45%, 61%, and 18% of the transplanted worms were recovered from the pulmonary arteries of dogs, cats, and rabbits, respectively, 1 and 3 mo later. The 5th-stage juvenile worms thus have the ability to re-migrate through tissue into the pulmonary arteries of the host.

Dirofilaria immitis is a heartworm that inhabits the right ventricle and pulmonary arteries of dogs and other Canidae. It is common in tropical, subtropical, and temperate areas of the world. *Dirofilaria immitis* develops into 3rd-stage infectious larvae (L_3) in a susceptible mosquito within approximately 2 wk of feeding on an infected dog; the L_3 then invade through the skin when the infected mosquito takes another canine blood meal. After invasion of the host, the developing larvae remain in the subcutaneous and fatty tissues and in sites under muscle membranes for approximately 3 mo. During this period, the 3rd and 4th larval molts occur at 3–6 days and 8–10 wk after infection, respectively (Kume et al., 1944a; Kume and Itagaki, 1955; Lichtenfels et al., 1985). The 5th-stage juvenile migrates via a small vein into the pulmonary arteries between 85 and 120 days after infection where it matures for 3 more months

(Kume et al., 1944a; Kume and Itagaki, 1955). Adult females produce microfilariae that are first detected in the circulation 6–7 mo after infection.

Kume et al. (1944b) and Kume and Itagaki (1955) reported the possibility of a re-migration phenomenon of 5th-stage juvenile worms to the pulmonary arteries when worms obtained from the pulmonary arteries of infected dogs are transplanted surgically into the subcutaneous tissues of uninfected dog.

In experimental infections of dogs with L_3 *D. immitis,* about 40% of the inoculated larvae reach the heart and pulmonary arteries as the final sites of infection (Hayasaki, 1982; Hayasaki and Ohishi, 1989a, 1989b). In experimental infections in cats, about 8% of inoculated L_3 reached these sites (Donahoe, 1975). In natural infections in cats, the average frequency of infection is lower, 1.9%, than in dogs within the same geographic regions (Rawlings and Calvert, 1995). However, in experimental infections of laboratory rodents with L_3 *D. immitis,* no successful infections have been reported.

Interestingly, experimental infections with adult *D. immitis* by surgical transfer to the heart via the jugular vein have been reported in dogs (Rawlings and McCall, 1985; Ohno et al., 1991). *Dirofilaria immitis* infection by surgical transfer in dogs has also been successful with 5th-stage juvenile worms removed from subcutaneous and fatty tissues (Kume et al., 1944b; Kume and Itagaki, 1955).

In the present study, live, 5th-stage juvenile *D. immitis* worms were collected from the pulmonary arteries of experimentally infected dogs and placed into sterilized saline for determining sex. Within a few minutes, the worms were surgically transplanted into subcutaneous sites of anesthetized, uninfected dogs, cats, and rabbits (Table I). For this procedure, the skin on an appropriate site of the back was surgically cut and a small hole was made in the subcutaneous areas under the skin using the end of a surgical knife. The worms were then inserted into the subcutaneous hole with a sterilized tweezer. Immediately after the insertion, the skin site was sutured without any antibiotic injections. The results show that surgically transplanted 5th-stage juvenile worms retain the capacity for tissue migration from subcutaneous tissue to the pulmonary arteries of dogs, cats, and even rabbits.

In these experiments, the mean percent recovery in 6 dogs was 45.3% (Table I). Recovery 1 mo and 3 mo after transplantation was essentially the same, except in 1 dog. This mean percent recovery is nearly the same as that in dogs infected via L_3 inoculation from mosquitoes.

The mean percent recovery in 7 cats was 60.9%. The 2 highest percent recoveries from cats (100% and 87.5%) were higher than from any of the 6 transplanted dogs, although there is no significant difference between the 2 groups.

In 10 rabbits, the mean percent recovery was 18.0%, signif-

TABLE I. Host adaptability of immature 5th-stage *Dirofilaria immitis* after subcutaneous transplantation.

Animal species	No. of worms transplanted, total (M/F)	Duration of donor infection	Duration of transplanted infection	No. of worms recovered			Gravidity‖
				Total (M/F)	Rate (%)	Mean (SD)(%)	
Dog 1	15 (ND)*	145	27	9 (2/7)	60.0	45.5 (15.2)†	−
2	15 (ND)	145	27	6 (3/3)	40.0		−
3	19 (10/9)	147	96	9 (3/6)	47.3		+#
4	20 (10/10)	147	96	10 (3/7)	50.0		+#
5	20 (10/10)	147	96	12 (5/9)	60.0		+#
6	20 (10/10)	147	96	3 (2/1)	15.0		+#
Cat 1	10 (5/5)	161	26	5 (3/2)	50.0	60.9 (21.9)‡	−
2	15 (ND)	145	27	6 (3/3)	40.0		−
3	15 (ND)	145	27	8 (4/4)	53.3		−
4	8 (ND)	139	30	7 (4/3)	87.5		−
5	7 (ND)	139	30	3 (1/2)	42.8		−
6	8 (ND)	139	30	8 (3/5)	100		−
7	7 (ND)	139	30	3 (0/3)	42.8		−
Rabbit 1	10 (5/5)	145	20	3 (1/2)	30.0	18.0 (13.2)§	−
2	10 (5/5)	145	23	4 (1/3)	40.0		−
3	10 (5/5)	145	28	2 (1/1)	20.0		−
4	10 (5/5)	145	28	2 (1/1)	20.0		−
5	10 (5/5)	145	28	2 (1/1)	20.0		−
6	10 (5/5)	145	28	3 (1/2)	30.0		−
7	10 (5/5)	145	28	2 (1/1)	20.0		−
8	10 (5/5)	147	96	0	0		−
9	10 (5/5)	147	96	0	0		−
10	10 (5/5)	147	96	0	0		−

* ND = not determined.
† vs. ‡ Not significant.
† vs. § Significant ($t = 3.56$, $P < 0.01$).
‡ vs. § Significant ($t = 4.72$, $P < 0.001$).
‖ Intrauterine microfilaria.
Positive with microfilaremia.

icantly lower than in the 6 dogs ($t = 3.56$, $P < 0.01$) or 7 cats ($t = 4.72$, $P < 0.001$), as calculated by Student's t-test (Table I). In these experiments, fewer worms were transplanted to avoid the risk of host mortality. The mean percent recovery in 7 rabbits 1 mo after transplantation was 25.7%. At 3 mo, no live worms were detected in 3 rabbits, but marked lung histopathology caused by dead worms was observed at necropsy.

The intrauterine microfilariae in all female *D. immitis* recovered were examined, but only the female worms recovered from 4 transplanted dogs (nos. 3–6) produced microfilaremia; none of the female worms recovered from 2 dogs, 7 cats, or 7 rabbits was gravid, indicating that these worms were still in an immature state.

The observed re-migration of 5th-stage juvenile *D. immitis* might possibly lead to the development of rabbit dirofilariasis. These data thus may provide an important means to clarify not only the migration or adaptation mechanism of the worm throughout development, but also the pathogenic mechanism of spontaneous thromboembolism and parenchymal lung disease. Therefore, this modified infection may contribute to the clarification of host–parasite relationships, as a laboratory animal model of human or domestic animal filariasis involving *D. immitis*.

LITERATURE CITED

DONAHOE, J. M. R. 1975. Experimental infection of cats with *Dirofilaria immitis*. Journal of Parasitology **61:** 599–605.

HAYASAKI, M. 1982. Reaginic and hemmaglutinating antibody production in dogs infected with *Dirofilaria immitis*. Japanese Journal of Veterinary Science **44:** 63–70.

———, AND I. OHISHI. 1989a. Influence of immune treatments against *Dirofilaria immitis* infection in dogs. Japanese Journal of Veterinary Science **51:** 540–546.

——— AND ———. 1989b. Influence of immunosuppressants against *Dirofilaria immitis* infection in dogs. Japanese Journal of Veterinary Science **51:** 955–960.

KUME, S. AND S. ITAGAKI. 1955. On the life-cycle of *Dirofilaria immitis* in the dog as the final host. British Veterinary Journal **111:** 16–24.

———, M. KOH, AND S. ITAGAKI. 1944a. Studies on the life cycle of *Dirofilaria immitis* (IX—III—1). Japanese Journal of Sogo Veterinary Science **1:** 342–350. [In Japanese.]

———, ———, AND ———. 1944b. Studies on the life cycle of *Dirofilaria immitis* (IX—III—2). Japanese Journal of Sogo Veterinary Science **1:** 379–389. [In Japanese.]

LICHTENFELS, J. R., P. A. PILITT, T. KOTANI, AND K. G. POWERS. 1985. Morphogenesis of developmental stages of *Dirofilaria immitis* (Nematoda) in the dog. Proceedings of the Helminthological Society of Washington **52:** 98–113.

OHNO, H., M. HAYASAKI, AND I. OHISHI. 1991. Determination of parasitic location of living adult *Dirofilaria immitis* and cardiac function of infected dogs as assessed by echocardiography. Journal of the Japanese Veterinary Medical Association **44:** 1115–1120. [In Japanese with English summary.]

RAWLINGS, C. A., AND C. A. CALVERT. 1995. Heartworm disease. *In* Textbook of veterinary internal medicine, S. J. Ettinger and E. C. Feldman (eds.). W. B. Saunders, Philadelphia, Pennsylvania, p. 1046–1068.

———, AND J. W. MCCALL. 1985. Surgical transplantation of adult *Dirofilaria immitis* to study heartworm infection and disease in dogs. American Journal of Veterinary Research **46:** 221–224.