肝蛭幼若虫のラット腹腔内移植による実験感染

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	佐伯, 英治 平詔, 亨 富下, 義文
巻/号	51巻1号
掲載ページ	p. 151-156
発行年月	1989年2月

Artificial Infection by Transplantation of Juvenile *Fasciola* Worms into the Abdominal Cavity of Rats

Hideharu SAEKI, Noriyuki TAIRA[1)], Yoshifumi TOMISHITA[2)], and Toshio ISHII

Department of Parasitology, Nippon Veterinary and Zootechnical College, Kyonan-cho, Musashino 180, [1)]Kyushu Branch Laboratory, National Institute of Animal Health, Chuzan-cho, Kagoshima 890-01, and [2)]Chuo Livestock Hygiene Service Center, Prefecture of Fukuoka, Hakata-ku, Fukuoka 816, Japan

(Received 19 April 1988/Accepted 6 October 1988)

ABSTRACT. Rats were successfully infected with Japanese *Fasciola* sp. by transplantation of juvenile worms (JW) or metacercariae (MC) into the abdominal cavity. Moreover, the rat was investigated on its suitability for different experiments with liver flukes. JWs or MCs transplanted intraperitoneally (IP) matured in the bile duct of rats. Moreover, more stable infections were established by inoculation of JWs than MCs. About 3 of 10–15 JWs transplanted into the abdominal cavity of a rat matured and laid eggs in the bile duct. The mean prepatent period was 63.5 days in the JW inoculated group. EPG values were kept constant at a level of 10^2–10^3 about 100 to 230 days after the transplantation of JWs. The life span of Japanese liver flukes was estimated to be about 400 days in rats. From these results, it was concluded that the rat is suitable for various experiments with *Fasciola* sp.—KEY WORDS: artificial infection, *Fasciola*, juvenile worm, transplantation.

Jpn. J. Vet. Sci. 51(1): 151–156, 1989

The surgical transplantation of helminths from donor to recipient hosts of various species has been reported. Hughes and Harness [7] first succeeded in transplantation of adult liver flukes from donor hosts to rats or rabbits.

When small laboratory animals are orally inoculated with *Fasciola* metacercariae (MC), both infective and recovery rates are often unexpectedly variable in general. The reasons may be attributed to the difficulty of determining the infectivity of MC by light microscopy alone, as described by Thorpe [17].

In order to produce more stable artificial infections, we tried to transplant juvenile *Fasciola* worms obtained from mice 2 weeks after inoculation with MCs into the abdominal cavity of recipient rats.

MATERIALS AND METHODS

Animals and metacercariae: Female SD rats and ddY mice were used in the experiments. Snail intermediate hosts, *Lymnaea ollula*, maintained in our laboratory were exposed to Japanese *Fasciola* miracidia hatched out from eggs originated from cattle slaughtered in Ibaraki Prefecture. One month later, MCs were recovered and stored at 4°C within 3 months until used for experiments.

Recovery of JWs and transplantation of JWs and MCs: Mice were orally given 30–50 MCs each and killed 2 weeks later to collect the liver. The livers were squashed gently with fingers in saline. The squashed liver mixtures were filtrated through a 100 mesh sieve of stainless steel. After filtrated out on the sieve, JWs were removed to sterile saline and washed 2–3 times. Vivid JWs were selected under a binocular microscope. Immediately after an incision about 5 mm long was made on the linea alba of the recipient rats under ether anesthesia, 10, 20 or 30 JWs kept in sterile saline were transplanted into the abdominal cavity through the incision with a Pasteur pipette.

On the other hand, as controls, 10, 20 or 30 MCs adhered onto a polyethylene sheet were transferred into the abdominal cavity of rats by the same technique as the above.

Experiment 1: Forty rats were divided into 2 groups of 19 and 21 animals, respectively. The first group was further subdivided into 3 groups of 7, 6 and 6 animals, respectively, which were intraperitoneally (IP) inoculated with 10, 20 and 30 JWs, respectively (JW group). The second group was also divided into 3 subgroups of 7 animals each, which were inoculated with 10, 20 and 30 MCs, respectively (MC group).

All the rats were killed from 3 to 7 weeks after the inoculation with JWs or MCs and their livers were removed. The livers were individually squashed and examined for juvenile worms under a binocular microscope.

Experiment 2: Fourteen rats were divided into 2 groups of 8 and 6 animals, respectively. The former group was further subdivided into 3 groups of 3, 2 and 3 animals, which were IP inoculated with 10, 20 and 30 JWs respectively. The latter group was divided into 3 subgroups of 2 animals each, which were IP inoculated with 10, 20 and 30 MCs, respectively. Fecal examination was carried out for excreted eggs by the slanted-rotation to the glass bead layer (SRGB) technique [13–15] every 2 or 3 days from 54 to 447 days after inoculation. All the rats except Nos. 2, 7 and 8 were killed at 120 (Nos. 12 and 14), 405 (Nos. 5 and 6), 433 (Nos. 1, 3 and 4) and 447 (Nos. 9, 10, 11 and 13) days after inoculation and examined for worms (Table 2).

RESULTS

Experiment 1: In the JW group, worms of different developmental stages were recovered from the liver parenchyma or bile duct of all the rats. The mean recovery rates were 44.3%, 55.8% and 53.9% in the groups inoculated with 10, 20 and 30 JWs, respectively. In the MC group, both infection and recovery rates were lower than those in the JW group. The infection rates were 1/7, 2/7 and 1/7, and the recovery rates were 1.4%, 2.9% and 0.5% in the groups inoculated with 10, 20 and 30 MCs, respectively (Table 1).

Experiment 2: As shown in Table 2, eggs were detected from all the rats of both groups. The mean prepatent period was 63.5 days (59–68 days) in the JW group and 58.7 days (55–65 days) in the MC group.

Table 1. Number of worms recovered from rats inoculated by transplantation of *Fasciola* juvenile worms or metacercariae

Group	Inoculum size	Number of worms recovered/rat (weeks after inoculation) 1	2	3	4	5	6	7	No. of rats used	No. of rats infected (%)	No. of worms transplanted	No. of worms recovered (%)
Juvenile worm	10	2	4	·	10	8	·	·	7	7 (100)	70	31 (44.3)
		1	3	·	3	·	·	·				
	20	7	12	·	17	·	·	·	6	6 (100)	120	67 (55.8)
		6	11	·	14	·	·	·				
	30	22	12	·	26	·	·	·	6	6 (100)	180	97 (53.9)
		12	12	·	13	·	·	·				
Metacercaria	10	·	·	0	0	0	1	·	7	1 (14.3)	70	1 (1.4)
		·	·	0	0	·	0	·				
	20	·	·	3	0	0	0	·	7	2 (28.6)	140	4 (2.9)
		·	·	1	0	·	0	·				
	30	·	·	0	0	0	1	·	7	1 (14.3)	210	1 (0.5)
		·	·	0	0	·	0	·				

No live worms were detected from 9 rats killed 405–447 days after inoculation, but fluke fragment-like matter was detected from 6 of them (Nos. 1, 3, 6, 10, 11 and 13). EPG values of all the JW group rats were almost constant at a level of 10^2-10^3 from about 100 to 230 days after inoculation. Then, EPG values decreased markedly in the rats except No. 4, 234–264 days after inoculation. Finally, EPG values declined to 0 in 3 rats, Nos. 3, 5 and 6, 354 days after inoculation (Fig. 1).

On the other hand, EPG values of the MC group rats were consistently lower than those of the JW group rats (Table 2).

DISCUSSION

Rabbits, guinea pigs, rats and mice are usually used for various kinds of experiments with *Fasciola* spp. [3, 5]. Of those animals, the rabbit is relatively expensive, especially for the primary screening test of anthelmintics, because rather spacious pens are required to keep the animals. In the guinea pig, liver flukes often migrate aberrantly to various parts of the body, so that, parasite recovery rates are not always definite when experiments are repeated. Since mice are highly susceptible to liver flukes, they often die in compliance with inoculum sizes in the course of experiments. Therefore, mice and guinea pigs may not be suitable for studying experimental chronic *Fasciola* infections.

Up to now, many immunological studies [16] and screening or evaluation tests of anthelmintics against the liver fluke [2, 3, 11] have generally been performed with rats. However, the important aspects in rat-liver fluke relationship have not always been elucidated in any detail [12], and Thorpe [17] indicated that the dosing technique and difference in the virulence of MCs used were the significant factors influencing the number of liver flukes present. There-

Table 2. Changes in EPGs number of worms recovered from rats inoculated by transplantation of *Fasciola* juvenile worms or metacercariae

Group	Rat No.	Inoculum size	Prepatent period (days after inoculation)	Time in days after inoculation when EPGs exceeded 100	Maximum EPGs	Findings at autopsy		
						EPGs[d]	No. of worms recovered	Days after inoculation
Juvenile worm	1	10	64[b]	71	1452	12	0	433
	2[a]	10	60	68	1231	—	3	244
	3	10	59	66	618	0	0	433
	4	20	64	67	1744	664	3	433
	5	20	63	69	1056	1	1	405
	6	20	68	88	1013	0	0	405
	7[a]	30	63	69	980	—	—	95
	8[a]	30	67	91	609	—	—	155
Metacercaria	9	10	58	124[c]	141	—	1	447
	10	10	55	71	108	0	0	447
	11	20	57	—	6	0	0	447
	12	20	65	—	50	—	1	120
	13	30	58	—	49	0	0	447
	14	30	59	—	13	—	1	120

a) Died on the day of autopsy.
b) In the JW group, the period includes 14 days in mice.
c) EPGs exceeded 100 only 124 days after inoculation.
d) EPGs at autopsy.

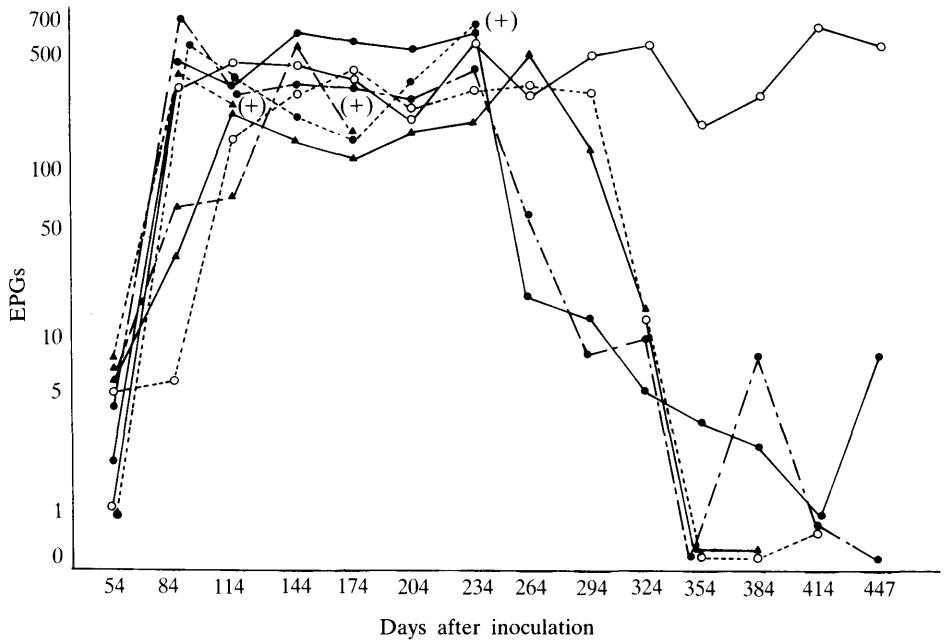

Fig. 1. Changes in EPGs in rats inoculated with juvenile worms (JWs) by transplantation
10 JWs transplanted (●); ●—● Rat No. 1, ●---● Rat No. 2 (+, died), ●-·-● Rat No. 3, 20 JWs (○); ○—○ Rat No. 4, ○---○ Rat No. 5, 30 JWs (▲); ▲—▲ Rat No. 6, ▲---▲ Rat No. 7 (+, died), ▲-·-▲ Rat No. 8 (+, died).

fore, the surgical transplantation of JWs or MCs was studied as a new method of artificial infection.

Krull and Jackson [10] already succeeded in infecting rabbits and sheep with juvenile *F. hepatica* obtained from mice and/or guinea pigs by surgical transplantation. Moreover, Hughes and Harness [7] tried to transplant adult *F. hepatica* into the abdominal cavity of rats and rabbits, and reported that transplanted worms can survive for at least 6–7 weeks in recipient rats. In addition, immunological studies were made on the rats inoculated with immature worms [6, 8] or MCs [9] of liver flukes by surgical transplantation. However, the fate of worms transplanted into rats is not always clarified in the previous reports.

In the present experiments, *Fasciola* JWs and MCs transplanted into rats matured and passed eggs in the bile duct. Compared with MCs, JWs showed the higher and more constant infection and recovery rates, because active JWs could be easily selected by their morphology and motility.

Lämmler [11] reported that 65–75% of the rats orally inoculated with 10 or 20 MCs survive and remain infected. On the other hand, Rajasakariah and Howell [12] inoculated rats orally with 1, 5, 10, 15, or 20 *F. hepatica* MCs and concluded that the proportion of MCs which develops to maturity is almost the same irrespective of the dose size. Thorpe [17] inoculated rats orally with varying numbers of *F. hepatica* MCs and reported that the number of worms recovered is the most stable in rats inoculated with 20 MCs, although rats inoculated with 80 and 160 MCs died.

In the present experiments, a maximum number of 26 young worms were harvested from the liver parenchyma 3–7 weeks after inoculation. However, since the bile duct of rats can harbor about 3 adult worms at

most, the surplus worms will be expelled from the bile duct sooner or later. Moreover, 2 of 3 rats inoculated with 30 JWs died in the relatively early stage of infection. These results indicate that a rat can be best inoculated with 10–15 or 20 JWs at most.

Mean prepatent period was 63.5 and 58.7 days in the JW and the MC groups, respectively. In the JW group, EPG values were kept at a constant level of 10^2–10^3 from about 100 to 230 days after inoculation, and thereafter they were declined gradually. In the MC group, however, EPG values were maintained at a considerably lower level than in the JW group probably owing to paucity of mature worms.

In 3 rats, Nos. 3, 5 and 6, EPG values were lowered to zero, 354 to 447 days after inoculation. No flukes were recovered from 3 rats, Nos. 1, 3 and 6. From these results, the life span of Japanese *Fasciola* sp. was estimated to be about 400 days in rats, although Alicata and Swanson [1] reported that most of adult *F. gigantica* are eliminated from cattle by the end of one year, but some of them might survive for at least three years and four months after infection.

In conclusion, rats are suitable for various experiments with *Fasciola* sp., because they can harbor about 3 adult worms each in the bile duct. Moreover, the experiments requiring fecal egg counts should be performed from about 100 to 230 days after inoculation.

REFERENCES

1. Alicata, J. E. and Swanson, L. E. 1941. Observations on the longevity of the liver fluke, *Fasciola gigantica*, in cattle. *Am. J. Vet. Res.* 2: 417–418.
2. Boray, J. C. 1969. Experimental Fascioliasis in Australia. pp. 95–210. *In*: Advances in Parasitology, vol. 7., Academic Press, London.
3. Boray, J. C., Happich, F. A. and Andrews, J. C. 1967. Studies on the suitability of the albino rat for testing anthelmintics activity against *Fasciola hepatica*. *Ann. Trop. Med. Parasitol.* 61: 104–111.
4. Dawes, B. 1962. On the growth and maturation of *Fasciola hepatica* L. in the mouse. *J. Helminthol.* 36: 11–38.
5. Dawes, B. and Hughes, D. L. 1964. Fascioliais: the invasive stages of *Fasciola hepatica* in mammalian hosts. pp. 97–165. *In*: Advances in Parasitology, vol. 2., Academic Press, London.
6. Doy. T. G. and Hughes, D. L. 1982. *In vitro* cell adherence to newly excysted *Fasciola hepatica*: failure to affect their subsequent development in rats. *Res. Vet. Sci.* 32: 118–120.
7. Hughes, D. L. and Harness, E. 1972. The experimental transfer of adult *Fasciola hepatica* between various mammalian host. *Res. Vet. Sci.* 13: 422–426.
8. Hughes, D. L. and Harness, E. 1973. The experimental transfer of immature *Fasciola hepatica* from donor mice and hamsters to rats immunized against the donor. *Res. Vet. Sci.* 14: 220–222.
9. Kelly, J. D., Campbell, N. J. and Dineen, J. K. 1980. The role of the gut in aquired resistance to *Fasciola hepatica* in rat. *Vet. Parasitol.* 6: 359–367.
10. Krull, W. H. and Jackson, R. S. 1943. Observations on the route of migration of the common liver fluke, *Fasciola hepatica*, in the definitive host. *J. Wash. Acad. Sci.* 33: 79–82.
11. Lämmler, G. 1959. Die Chemotherapie der Fasciolose. III. Mitteilung über die experimentelle *Fasciola hepatica*-Infektion der Albino-Ratte. *Z. Tropenmed. Parasitol.* 10: 379–384.
12. Rajasakariah, G. R. and Howell, M. J. 1977. *Fasciola hepatica* in rats: Effect of age and infective dose. *Int. J. Parasitol.* 7: 119–121.
13. Taira, N., Ueno, H., Kawano, M. and Tsunokawa, A. 1979. New sieving techniques with the glass bead layer for the detection of *Fasciola* eggs from cattle feces. 3. Evaluation as methods of practical field fecal examination and quantitative egg count. *Jpn. J. Parasitol.* 28: 15–19. (in Japanese with English summary).
14. Taira, N., Yoshihara, S. and Ueno, H. 1978. New sieving techniques with the glass bead layer for the detection of *Fasciola* eggs from cattle feces. 1. Procedures and preliminary tests on the vibration technique. *Jpn. J. Parasitol.* 27: 191–195. (in Japanese with English summary).
15. Taira, N., Yoshihara, S., Ueno, H., Yoshihara, T. and Iwase, K. 1978. New sieving techniques with the glass bead layer for the detection of *Fasciola* eggs from cattle feces. 2. Preliminary tests on the slanted rotation technique. *Jpn. J. Parasitol.* 27: 387–391. (in Japanese with English summary).
16. Taylor, M. G. 1980. Vaccination against trematodes. pp. 115–140. *In*: Vaccines against Parasites. Symp. Br. Soc. Parasitol. vol. 18. (Taylor, A. E. R. and Muller, R. eds.), Blackwell Sci.

Publ., Oxford.
17. Thorpe, E. 1965. Liver damage and the host-parasite relationship in experimental fascioliasis in the albino rat. *Res. Vet. Sci.* 6: 498–509.

要　　約

肝蛭幼若虫のラット腹腔内移植による実験感染：佐伯英治・平　詔亨[1]・富下義文[2]・石井俊雄（日本獣医畜産大学獣医寄生虫学教室，[1]農林水産省家畜衛生試験場九州支場，[2]福岡県中央家畜保健衛生所）――肝蛭人工感染法の１つの試みとして，日本産肝蛭（*Fasciola* sp.）幼若虫（JW）およびメタセルカリア（MC）をラットの腹腔内に移植した．その結果，移植されたJWおよびMCの一部は胆管に至り，成虫になることが明らかとなった．しかし，寄生率および虫体回収率の何れも前者が優り，JWを10～15匹移植した場合約３匹の成虫が回収された．JW移植群のprepatent periodは平均63.5日であり，移植後100～230日の間EPGは10^2～10^3の値を維持していた．移植虫体のラット体内における生存期間は約400日であった．これらの成績から，ラットは種々の肝蛭感染実験に十分供試し得る動物であると考えられる．