

Bruce Z. Lang

Department of Biology
Eastern Washington State College
Cheney, Washington

Experimental Infections with *Fasciola hepatica* from Eastern Washington and Northern California¹

The laboratory mouse is a suitable experimental host for *Fasciola hepatica* L. according to Dawes (1962), Dawes and Hughes (1964), Lang (1966, 1967, 1968a,b), and Boray (1963); however, great variation was reported in the size of the infecting dose, worm recovery, and mortality in mice. Studies by Shirai (1927), Taylor and Parfitt (1957), and Lagrange and Gutmann (1961) indicated that the laboratory mouse was not a suitable experimental host as a single parasite might result in death of the host. Apparently, the strain of mouse and parasite play a role in the suitability of any *Fasciola*-mouse system for experimental work.

Studies on the immunology and host-parasite relationships of *F. hepatica* in a single strain of mouse have demonstrated that this strain is suitable for studying certain host responses manifested against two-worm infections and that males develop acquired immunity following immunizing infection (Lang, 1966, 1967, 1968a,b; Lang *et al.*, 1967). This work used a strain of parasite from northern California alternately maintained in mice, rabbits, and occasionally rats in an attempt to avoid any immediate or long-term adaptation to the mouse host. The snail *Lymnaea columella* Say was utilized in all published studies; however utilizing *L. bulimoides* Lea had no detectable effect on host and parasite behavior following single two-worm infections (Lang, unpublished data).

The present study deals with the host responses and parasite growth and behavior of *Fasciola* from eastern Washington. Before immunological studies on the *Fasciola*-mouse system could be continued using a parasite from a different area, the host responses manifested against this parasite and parasite behavior had to be determined.

Materials and Methods

Mice utilized were from an isologous strain maintained in the Department of Parasitology, University of North Carolina. Techniques for infection and necropsy of mice, handling of recovered worms, determination of host responses, and infection of snail hosts have been described (Lang, 1966). Tissues were processed using an International Cryostat.

Eggs of *Fasciola* from eastern Washington were obtained from infected cattle, while eggs from California were obtained from laboratory-infected rabbits. Eggs were embryonated and one- to four-week-old laboratory-raised *L. bulimoides* were exposed to miracidia. Twenty-four hours after exposure, snails were transferred to clay pots (22 cm by 5 cm) containing food and filtered spring water. Sixty days after exposure snails were isolated in jars for collection of metacercariae which were stored at 13°C. Worm measurements are in millimeters, the mean followed by the range.

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Experimental Procedures and Results

To determine baseline data on *Fasciola* from eastern Washington, 56 male mice with an average weight of 31.5 g at infection (18 to 20 weeks old) were divided into two groups of 36 (Group I, Washington) and 20 (Group II, California) and each mouse was given two metacercariae (40 to 80 days old). Body weights were taken the day of infection, 7, 14, 18, 22, 25, 27, 31, 35, 42, and 49 days after infection. Four mice from each group were used for differential leukocyte determinations at the above intervals. Group III (uninfected controls), 12 mice, provided normal body weights and differential counts. For Group I, eight mice were necropsied at 10, 12, 17, 19, 24, 33, 35, and 40 days post-infection for worm recovery and tissue for histopathology. Remaining mice were necropsied 50 days post-infection. Group II mice were necropsied at corresponding times after 19 days of infection and at selected mortality times for Group I. Thus, for Group II 16 mice were necropsied at 24, 30, 32, 40, and 50 days post-infection. No tissues for histopathology were taken from this group as tissue responses for this system are well documented (Lang, 1966, 1967). Recovered worms from both groups were measured at selected times after infection.

All mice exposed to metacercariae were infected. For Group I, 90.3 percent (65/72) of the worms were recovered, while 90.0 percent (36/40) of the worms were recovered from mice of Group II. Group I mice began dying 25 days after infection. Between 25 and 33 days after infection 10 animals died (Table 1). Five animals had been necropsied prior to the onset of mortality, thus they were not mortality risks. Mortality in Group I was 32.3 percent (10/31). Group II mice began dying 26 days after infection. Between 26 and 28 days, four mice died. Two animals had been necropsied prior to the onset of mortality. Mortality in Group II was 22.2 percent (4/18).

TABLE 1. Worm recovery and location of worms from mice infected with two *Fasciola hepatica* metacercariae each at intervals after infection.

Days after infection	Number of Mice Group I	Number of Mice Group II	Location and Condition Group I	Condition Group II
10-19	4(8) ¹	0	LP-I ²	—
24	1(2)	2(3)	LP-I	LP-I
25	4*(7)	0	LP-I	—
26	2*(3)	1*(2)	LP-I	LP-I
27	1*(1)	2*(4)	LP-I	LP-I
28	0	1*(2)	—	LP-I
29	1*(2)	0	LP-I	—
30	1*(2)	2(4)	LP-I	LP-I
32	1*(2)	2(4)	BD-I ³	LP-I
33	1(1)	2(4)	BD-M ⁴	LP-I
35	1(2)	2(4)	BD-M	BD-I
40	1(1)	2(3)	BD-M	BD-M
50	18(34)	4(6)	BD-M	BD-M
Totals	36(65)	20(36)		

¹ Number of worms recovered ().

² Liver parenchyma, immature.

³ Common bile duct, immature.

⁴ Common bile duct, mature.

* Animal(s) died.

As shown in Table 1, worms were located in the liver parenchyma of mice from both groups until 30 days after infection. In Group I, immature worms were recovered from the common bile duct by 32 days post-infection, while immature worms from Group II were not recovered from the common bile duct until 35 days post-infection. Worms of Group I were producing few eggs by 33 days after infection and numerous eggs by 35 days after infection, while worms of Group II did not produce eggs until 40 days after infection and then only a few. By 50 days these worms were producing numerous eggs.

Prior to movement into the common bile ducts worms of Group I were slightly larger than those of Group II. Group I: 24 days, 4.4 (4.3 to 4.5); 26 days, 4.8 (4.6 to 5.0); 30 days, 6.1 (5.9 to 6.3); 32 days, 6.8 (6.7 to 6.9); 35 days, 7.2 (7.0 to 7.4); 40 days, 12.6 (12.6); 50 days, 13.8 (13.5 to 14.3). Group II: 24 days, 3.6 (3.3 to 4.0); 26 days, 4.2 (4.0 to 4.4); 30 days, 5.4 (4.9 to 5.7); 32 days, 6.7 (6.4 to 6.9); 35 days, 8.8 (8.2 to 9.4); 40 days, 12.7 (12.4 to 13.0); 50 days, 12.9 (12.4 to 13.0). In both groups, after the worms reached the common bile duct and began to produce eggs, there was a decrease in growth rate. This was more marked in worms of Group II.

Host responses for mice of both groups were very similar. Between 18 and 25 days after infection mice from Group I lost an average of 2.5 g per animal while mice from Group II lost an average of 2.0 g during the same interval. Both groups gained weight after 25 days with Group I mice averaging 33.1 g by 42 days after infection and Group II mice averaging 32.9 g at the same time. Group I mice gained weight somewhat more rapidly. Uninfected controls (Group III) showed steady weight gain throughout the experiment.

The differential leukocyte determinations for Group I indicated a reversal in the lymphocyte-neutrophil ratio from 22 to 35 days post-infection as compared to a corresponding reversal occurring 25 to 31 days post-infection in Group II. No reversal was observed in mice of Group III.

Host reaction to worm damage in the liver of Group I mice at 10 to 17 days post-infection was characterized by an inflammatory zone of polymorphonuclear leukocytes and necrotic hepatic cells around worm burrows. By 17 days after infection large areas of the parenchyma were necrotic and small lymphocytes were present throughout the liver. This pathology and that seen after 19 days of infection was almost identical with previous reports for this system (Lang, 1966, 1967).

Discussion

In comparing the two parasite populations it is apparent that differences in worm behavior are present. Worms of the Washington population migrated into the common bile duct sooner (three days), produced eggs earlier (seven days), and were larger prior to moving into the common bile duct and by 50 days after infection than worms of the California population. Also, mortality was higher (32.3 vs. 22.2 percent) in mice infected with worms from Washington. It was also observed that overall gross liver damage appeared more severe in these mice than seen in Group II and previous work (Lang, 1966).

The histopathological and weight course of the infections appears to be similar between the two populations, except that mice infected with worms from the California population did not appear to gain weight as rapidly after the acute phase of

infection. Slight differences were observed in the period of lymphocyte-neutrophil inversion. Mice of Group I had a longer reversal period (22 to 35 days post-infection) which was initiated three days sooner than in mice in Group II. It appears that the worms from the Washington population are slightly more pathogenic in the strain of mouse used than worms from the California population as seen in this study and others (Lang, 1966, 1967, 1968a).

With the above similarities in host responses and slight differences in parasite biology, it is felt that the experimental approach previously used in the mouse-*Fasciola* system and the data accumulated on host-parasite relationships and acquired immunity are applicable to this system using *F. hepatica* from Washington.

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Literature Cited

- Boray, J. C. 1963. Standardization of techniques for pathological and anthelmintic studies with *Fasciola* spp. Proc. 1st. Int. Con. World Assoc. Adv. Vet. Parasit., Hanover. Pp. 34-45.
- Dawes, B. 1962. On the growth and maturation of *Fasciola hepatica* in the mouse. J. Helm. 36: 11-38.
- _____, and D. L. Hughes. 1964. Fascioliasis: the invasive stages of *Fasciola hepatica* in mammalian hosts. In Ben Dawes (ed.), Advances in Parasitology, Vol. II. Academic Press, London.
- Lagrange, E., and A. Gutmann. 1961. Sur l'infestation experimentale de la souris par *Fasciola hepatica*. Riv. Parasit. Rome 22: 93-101.
- Lang, B. Z. 1966. Host-parasite relationships of *Fasciola hepatica* in the white mouse. I. Response to a primary infection. J. Elisha Mitchell Sci. Soc. 82: 195-203.
- _____. 1967. Host-parasite relationships of *Fasciola hepatica* in the white mouse. II. Studies on acquired immunity. J. Parasit. 53: 21-30.
- _____. 1968a. Acquired immunity by *Fasciola hepatica* in the laboratory white mouse. Am. J. Trop. Med. Hyg. 17: 561-567.
- _____. 1968b. Host-parasite relationships of *Fasciola hepatica* in the white mouse. III. Worm transfer. Proc. Okl. Acad. Sci. 47: 81-84.
- _____, Larsh, J. E., Jr., M. F. Weatherly, and H. T. Goulson. 1967. Demonstration of immunity to *Fasciola hepatica* in recipient mice given peritoneal exudate cells. J. Parasit. 53: 208-209.
- Shirai, M. 1927. The biological observations on the cysts of *Fasciola hepatica* and the route of migration of young worms in the final host. Sci. Rep. Gov. Inst. Inf. Dis. Tokyo 6: 511-523.
- Taylor, E. L., and J. W. Parfitt. 1957. Mouse test for infectivity of metacercariae with particular reference to metacercariae in snail feces. Tr. Am. Micro. Soc. 76: 327-328.

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